Abiotic and Biotic Stress Tolerance in Wheat: the Role of Arbuscular Mycorrhizal Fungi.

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TABLE OF CONTENTS

Chapter *Page*

Dedication ..... **v**

Acknowledgements ..... **xii**

Abstract ..... **xiii**

Chapter 1. Literature Review. **1**

1.1. Introduction **1**

1.2. Mycorrhizae **1**
Mycorrhizal-Host Relationships **1**
Taxonomy-Phylum Glomeromycota **6**
Gigaspora margarita **6**
Glomus intraradices **7**

1.3. Macronutrient Uptake in Mycorrhizal Plants **7**
Phosphorus **7**
Nitrogen **8**
Potassium **9**

1.4. Allelopathy **9**

1.5. Sorghum - *Sorghum bicolor* (L.) Moench **10**

1.6. Wheat - *Triticum aestivum* (L.) **11**

1.7. Insect-plant interactions **11**
Fall armyworm (FAW) **11**
Aphids **12**
Aphid Predators **15**
Aphid pheromones **16**

1.8. Alterations in host physiology by AMF Colonization **18**
Alterations in host chemistry **18**
Protection against insects **19**
Protection against pathogens **20**

1.9. Research goals **21**

Chapter 2. Material and Methods **22**

2.1. Mycorrhizal (AMF) treatments **22**

2.2. Chemical materials **22**

2.3. Plant materials and growth conditions **22**

2.4. AMF inoculation and assessment **23**

2.5. The effect of mycorrhizae on allelopathy **24**
Experimental Design **24**
Shoot growth **24**
Root weights **24**
Chlorophyll determination **24**
Percentage survival **25**
Statistical analysis **25**

2.6. The effect of mycorrhizae on aphid attraction **25**
No-choice experiments **25**
Choice experiments **25**
Data analysis **26**

2.7. The effect of mycorrhizae on fall armyworm (FAW) feeding **27**
Spodoptera frugiperda cultures **27**
No-choice Experiments **27**
Choice Experiments. All treatments **27**
Pairwise comparison **28**
Fall armyworm variables **28**

2.8. The effect of mycorrhizae on seedling disease caused by *Bipolaris sorokiniana* .. **28**
Experimental Design **28**
Bipolaris sorokiniana culture **28**
Seedling disease rating scale **29**

Chapter 3. Result **31**

3.1. The effect of mycorrhizae on allelopathy (*Sorghum bicolor*) **31**

3.2. Effect of mycorrhizae on allelopathy (sorghum x Sudangrass hybrid) **44**

3.3. Effect of mycorrhizae on aphid attraction **56**
Natural Infestation **56**
Choice tests **57**
No-choice tests **62**

3.4. Fall armyworm (Spodoptera frugiperda) leaf assays **65**
Choice experiment (All treatments) **65**
Choice experiment (pairwise comparison) **72**
No-choice experiment **77**

3.5. Effect of mycorrhizae on seedling disease caused by *Bipolaris sorokiniana* **78**

Chapter 4. Discussion **87**

Chapter 5. Summary **93**

Cited Reference **95**

VITA **111**

Appendices **112**

LIST of TABLES

Table *Page*

Table 2.1. Rating scale used to evaluate wheat seedling disease caused by *Bipolaris sorokiniana*.....30

Table 3.1. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings in sorghum allelopathy trials 31

Table 3.2. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings in allelopathy trials (sorghum x Sudangrass hybrid).....44

Table 3.3. Number of aphids recorded in natural experiment of wheat seedlings 57

Table 3.4. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings used in choice tests 58

Table 3.5. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings.....62

Table 3.6. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings used in fall armyworm feeding assays.....65

Table 3.7. Effect of mycorrhizae on fall armyworm (*Spodoptera frugiperda*) feeding; values are *P*-values for a F-protected LSD 72

Table 3.8 Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 6-week-old wheat seedlings used in seedling disease assays.....78

Table A.1. Statistical value for experiments on 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*).....112

Table A.2. Statistical values for experiments on 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained a Sorghum x Sudangrass hybrid 113

Table A.3. Statistical values for experiments on 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*) and infested with bird cherry-oat aphid (*Rhopalosiphum padi*).....114

Table A.4. Statistical values for experiments on leaves (Trial A) collected from 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*).....114

Table A.5. Statistical values for experiments on leaves (Trial B) collected from 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*).....115

Table A.6. Statistical values for choice tests (pairwise comparisons) on the numbers of fall armyworm (*Spodoptera frugiperda*) larvae feeding on leaves collected from 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*). 116

Table A.7. Statistical values for choice tests (pairwise comparisons) on feeding estimates of fall armyworm (*Spodoptera frugiperda*) larvae feeding on leaves collected from 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*). 117

Table A.8. Statistical values for experiments on 6-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*), and inoculated with or without *Bipolaris sorokiniana*.....118

LIST of FIGURES

Figure *Page*

Fig. 3.1. Effect of mycorrhizae on fresh shoot weight (g) (control) 33
Fig. 3.2. Effect of mycorrhizae on fresh shoot weight (g) (without control) 34
Fig. 3.3. Effect of mycorrhizae on stem diameter (mm) (control) 35
Fig. 3.4. Effect of mycorrhizae on stem diameter (mm) (without control) 36
Fig. 3.5. Effect of mycorrhizae on fresh root weight (g) (control) 37
Fig. 3.6. Effect of mycorrhizae on fresh root weight (g) (without control) 38
Fig. 3.7. Effect of mycorrhizae on dry root weight (g) (control) 39
Fig. 3.8. Effect of mycorrhizae on dry root weight (g) (without control) 40
Fig. 3.9. Effect of mycorrhizae on chlorophyll A and B (µg/mL) 41
Fig. 3.10. Effect of mycorrhizae on concentration of total chlorophyll (A+B) 42
Fig. 3.11. Effect of mycorrhizae on chlorophyll A to Chlorophyll B (A/B) 43
Fig. 3.12. Effect of mycorrhizae on shoot height (cm) (control) 45
Fig. 3.13. Effect of mycorrhizae on shoot height (cm) (without control) 46
Fig. 3.14. Effect of mycorrhizae on fresh shoot weight (g) (control) 48
Fig. 3.15. Effect of mycorrhizae on fresh shoot weight (g) (without control) 49
Fig. 3.16. Effect of mycorrhizae on stem diameter 50
Fig. 3.17. Effect of mycorrhizae on fresh root weight (g) 51
Fig. 3.18. Effect of mycorrhizae on dry root weight (g) (control) 52
Fig. 3.19. Effect of mycorrhizae on dry root weight (g) (without control) 53
Fig. 3.20. Effect of mycorrhizae on chlorophyll A and B (µg/mL) 54
Fig. 3.21. Effect of mycorrhizae on concentration of total chlorophyll (A+B) 55
Fig. 3.22. Effect of mycorrhizae on the ratio of chlorophyll A to Chlorophyll B 56
Fig. 3.23. Effect of mycorrhizae on survival of wheat seedlings used in choice tests .. 59
Fig. 3.24. Effect of mycorrhizae on aphid numbers on wheat seedlings (choice experiment) 60
Fig. 3.25. Effect of mycorrhizae on volatiles of wheat (choice test) 61
Fig. 3.26. Effect of mycorrhizae on plant survival, plant height (cm), and wheat weight (g) in no-choice test 63
Fig. 3.27. Effect of mycorrhizae on aphid number of wheat in no-choice experiment 64

Fig. 3.28. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) – subjective estimate of leaf damage 66
Fig. 3.29. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test)- subjective estimate of leaf damage (without control) 67
Fig. 3.30. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test)- image analysis of leaf damage (control) 68
Fig. 3.31. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) – image analysis of leaf damage (without control) 69
Fig. 3.32. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) –rating scale (control) 70
Fig. 3.33. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) –rating scale (without control) 71
Fig. 3.34. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) – subjective estimate of leaf damage 73
Fig. 3.35. Effect of mycorrhizae on fall armywormfeeding on wheat leaves (choice test) - image analysis of leaf damage 74
Fig. 3.36. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) – subjective estimate of leaf damage 75
Fig. 3.37. Effect of mycorrhizae on fall armywormfeeding on wheat leaves (choice test) – surviving larvae 76
Fig. 3.38. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) – non-feeding larvae 77
Fig. 3.39. Effect of mycorrhizae on plant survival (%) 80
Fig. 3.40. Effect of mycorrhizae on shoot height (cm) 81
Fig. 3.41. Effect of mycorrhizae on fresh shoot weight (g) 82
Fig. 3.42. Effect of mycorrhizae on dry shoot weight (g) 83
Fig. 3.43. Effect of mycorrhizae on fresh root weight (g) 84
Fig. 3.44. Effect of mycorrhizae and pathogen on fresh root weight (g) 85
Fig. 3.45. Effect of mycorrhizae on disease rating (1-6) - subjective rating scale 86
Fig. A.1. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) - subjective estimate of leaf damage (without control) 120

Fig. A.2. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) –rating scale (control) **121**
Fig. A.3. Effect of mycorrhizae on larvae of fall armyworm on wheat leaves (choice test- pairs) (control × *Gi*) **122**
Fig. A.4. Effect of mycorrhizae on fall armywormfeeding on wheat leaves (choice test) –rating scale (control × *Gi*) **123**
Fig. A.5. Effect of mycorrhizae on larvae of fall armyworm on wheat leaves (choice test) (NM × *Gi*) **124**
Fig. A.6. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) – subjective estimate of leaf damage (NM × *Gi*) **125**
Fig. A.7. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test)- image analysis of leaf damage (NM × *Gi*) **126**
Fig. A.8. Effect of mycorrhizae on larvae of fall armyworm on wheat leaves (choice test) (control × *Gm*) **127**
Fig. A.9. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test)- image analysis of leaf damage (control × *Gm*) **128**
Fig. A.10. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) –rating scale (control × *Gm*) **129**
Fig. A.11. Effect of mycorrhizae on larvae of fall armyworm on wheat leaves (choice test) (control × NM) **130**
Fig. A.12. Effect of mycorrhizae on fall armywormfeeding on wheat leaves (choice test) – subjective estimate of leaf damage (control × NM) **131**
Fig. A.13. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test)- image analysis of leaf damage (control × NM) **132**
Fig. A.14. Effect of mycorrhizae on fall armywormfeeding on wheat leaves (choice test) –rating scale (control × NM) **133**
Fig. A.15. Effect of mycorrhizae on larvae of fall armyworm on wheat leaves (choice test) (NM× *Gi*) **134**

Fig. A.16. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) – subjective estimate of leaf damage (NM× *Gi*) 135
Fig. A.17. Effect of mycorrhizae on fall armywormfeeding on wheat leaves (choice test)- image analysis of leaf damage (NM× *Gi*).....136
Fig. A.18. Effect of mycorrhizae on fall armyworm feeding on wheat leaves (choice test) –rating scale (NM× *Gi*).....137
Fig. A.19. Effect of mycorrhizae on fall armyworm survivorship of wheat leaves (no-choice test) (control).....138
Fig. A.20. Effect of mycorrhizae on fall armyworm survivorship on wheat leaves (no-choice test) – without control 139
Fig. A.21. Effect of mycorrhizae on on wheat leaves (no-choice) – subjective estimate of leaf damage (control).....140
Fig. A.22. Effect of mycorrhizae on fall armyworm on wheat leaves (no-choice) – subjective estimate of leaf damage (without control) 141
Fig. A.23. Effect of mycorrhizae on fall armyworm on wheat leaves (no-choice) – image analysis estimate of leaf damage.....142
Fig. A.24. Effect of mycorrhizae on fall armyworm on wheat leaves (no-choice)- image analysis estimate of leaf damage (without control).....143
Fig. A.25. Effect of mycorrhizae on fall armyworm on wheat leaves (no-choice test) – subjective rating scale (control).....144
Fig. A.26. Effect of mycorrhizae on fall armyworm on wheat leaves (no-choice test) – subjective rating scale (without control)……..…145

Dedication

This thesis is dedicated to my beloved parent, Mansour and Ghlia, who taught me to never give up irrespective of the obstacles and hurdles. I am so grateful to my best friend, Salheen, who always encouraged me during the difficult times. Sadly, he died during the liberation of Libya.

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Although my family and my wife, Somia, were not physically with me, their presence always inspired and motivated me to complete this document.

Abstract

Rhizospheres of crop plants are complexes of chemical and microbial interactions. Many plants produce allelochemicals, substances that inhibit growth of other plants and microorganisms. In previous research, colonization of *Echinacea purpurea* by beneficial mycorrhizal fungi appeared to alleviate the effects of allelochemicals on the growth and the development of the medicinal herb. The overall aims of the work reported here were to determine if colonization by arbuscular mycorrhizal fungi (AMF) alters responses of common wheat (*Triticum aestivum*) to stress caused by abiotic factors [allelopathic effects of two sorghum hosts (*Sorghum bicolor* and a sorghum x Sudangrass hybrid)] and biotic agents [bird-cherry oat aphid (*Rhopalosiphum padi*) (BCOA) and *Bipolaris* species (*Bs*)] stress. In all greenhouse experiments, wheat seeds were planted into each of four treatments: 1) control (no-mycorrhizae, no-sorghum); 2) NM (no-mycorrhizae, sorghum); 3) *Gm* (AMF, *Gigaspora margarita*, previously propagated on sorghum); and 4) *Gi* (AMF, *Glomus intraradices*, previously propagated on sorghum). Sorghum allelopathy was not alleviated by AMF colonization. In all studies, control wheat plants had greater biomass (e.g., fresh shoot and root weight) than plants in all other treatments. Furthermore, biomass of wheat seedlings colonized with AMF (either *Gm* or *Gi* treatments) was not different from plants grown after sorghum but without mycorrhizae (NM). In two natural insect infestations, mycorrhizal plants were less preferred by *R. padi* than non-mycorrhizal (NM) plants or control plants. However, in choice and non-choice aphid studies, this preference was not found among the treatments. Treatment had no effect on larval feeding behavior of fall armyworm (*Spodoptera frugiperda*) on wheat leaves in laboratory assays. In growth chamber studies, inoculation with *Bs* had no effect on disease rating or growth of mycorrhizal and NM wheat seedlings. Our results indicate that AMF are not effective agents for control of abiotic (sorghum allelopathy) or biotic (herbivory by BCOA or reduction of plant vigor caused by *Bs*) stress; however variability in all studies was high so further research is needed before their use for these purposes is dismissed.

Key words. Mycorrhizae, allelopathy, *Triticum aestivum*, *Sorghum bicolor*, *Bipolaris*, *Rhopalosiphum padi*, *Spodoptera frugiperda*.

Chapter 1

Literature Review

1.1. Introduction

Plants have evolved symbiotic relationships with partner organisms (e.g., fungi, bacteria, insects). The term symbiosis has been defined as two living organisms that live and interact together (Varma and Hock, 1994). There are many types of symbiosis that have been well documented and studied; these are both parasitic and mutualistic ones. In the parasitic interactions, only one partner benefits from this association; the other partner is harmed. In mutualistic interactions, both partners receive benefits; there is no superiority of one organism above the other.

Mutualism is typically the rule in plants (i.e., in their native ecosystem, most, if not all, plants have mutualistic partners), and there are many examples of beneficial interactions between plants and microbial symbionts (fungi, bacteria, or both). The mutualism between plant and fungal symbionts is based upon bidirectional benefits to both partners; the fungus provides the plant with many advantages such as increased nutrition and resistance to plant pathogens (e.g., fungi, bacteria, nematodes, and viruses) (Newsham, 1994; Harrier and Watson, 2004; Smith and Read, 2008), and in return, the plant sustains the fungus. Mutualistic fungi typically derive carbohydrates from the plant because they lack photosynthetic capability.

1.2. Mycorrhizae

Mycorrhizal-Host Relationships. Mycorrhizal colonization evolved by the Early Devonian era, approximately 400 hundred years ago. In the fossil record, plants colonized by mutualistic fungal partners identified as arbuscular mycorrhzial fungi have been identified. In Rhynie Chert, Scotland, one of the richest sites for ancient fossils, a vascular plant *Nothia aphylla* Lyon ex El-Saadaya et Lacey was colonized with a group of *Glomus*-like fungi. Both non-spetate hyphae and spores were found. There is not universal agreement that these are AMF structures because the physiological functions of

those fossils could not be tested, and acidic treatments implemented to recover the fossils altered morphology making it hard to compare them with the well-known present AMF structures (Redecker et al., 2000; Bonfante and Genrea, 2008; Pirozynski and Malloch, 1975). It is hypothesized that terrestrial plants in their early stages of life did not have true roots so they depended upon a symbiotic relationship with fungi; this helped them to establish their root systems in very harsh environments. Plants have coevolved over time to decrease their dependence on their fungal partners, but mycorrhizal relationships are still prevalent in the plant kingdom.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil inhabitants that form symbiotic associations with root systems of most plant species. Although most plants (ca. 80%) including angiosperms, gymnosperms, pteridophytes, and a few bryophytes are colonized with AMF, no members of at least three plant families (Brassicaceae, Caryophyllace, and Chenopodiaceae) are colonized by AMF (Smith and Read, 2008); most plants classified in these families contain high concentrations of antifungal compounds. The AMF are divided into two main types based on morphological traits – the Arum-type and Paris type. Their names are derived from the names of the plants on which they were originally described, *Arum maculatum* L. and *Paris quadrifolia* L. The AMF are classified based on the type of hyphal penetrations into the plant cell. In the *Arum* type, hyphae grow intercellularly in the cortex and form arbuscules within the plant cell; this type is considered to be "typical arbuscular formation." In contrast, in the Paris type the hyphae grow intracellularly in the cortex to form arbuscules, and this type occurs less frequently in nature than the former (Smith and Read, 2008; Smith et al., 2001).

Although AMF cannot be propagated in the absence of a plant host, the fungi can grow and produce limited mycorrhizal structures when there is no compatible interaction between the fungus and the host (i.e., non-host plant). Restricted mycorrhizal structures, such as little branching hyphae, last for a short period of time. Duration of hyphal survival depends upon the type of mycorrhizal species, environmental conditions, and host factors (Requena et al., 2007). The question of why AMF cannot be cultured, however, still remains unanswered. One hypothesis is that AMF lacked the ability to

replicate DNA outside appropriate plant host tissues (Burggraaf and Beringer, 1989); however, more recent studies have documented DNA synthesis and reproduction by AMF nuclei in the absence of the host (Bécard and Pfeffer, 1993). Another hypothesis is that AMF have no carbon fixation abilities, due to their complete dependence on the host plant to supply the needed carbon (Gadkar et al., 2001). More research is needed in order to understand growth and development of these organisms.

In the presence of the host, spores or any source of mycorrhizal inocula, (such as roots infected with hyphae) germinate/activate, and produce a final mycelium more rapidly than in the presence of a non-host plant. Signals for host recognition initiate changes in gene expression that result in the induction of strigolactone and its derivatives, release of lipophilic compounds, and induction/ release of unknown compounds that stimulate the fungal development (Tamasloukht et al., 2003). Not only do these metabolic signals promote hyphal progression, but they also induce full utilization of the spore lipid and nutrient reservoirs, and support the growth of tissues (i.e., hyphae and appressoria). Rhizobial bacteria require flavonoids as recognition and stimulation factors. Since these symbiotic relationships have many similarities with AM, flavonoids were thought to be involved in AMF host recognition and symbiosis, but recent research indicates that flavonoids do not play a huge role in AMF colonization. Maize mutants that were impaired in flavonoid production were colonized by mycorrhizae at the same rate and to the same extend as wild type (Buee et al., 2000; Becard et al., 1995).

In addition to the host factors that regulate AMF spore germination, there are other important factors including both environmental and edaphic factors that control the process of germination. Other factors include: pH, CO_2, temperature, mineral and organic nutrients, and moisture. Some of these factors have a great impact on germination; however, others have less impact. Mycorrhizal spores vary in their response to pH. Spores of *Gigaspora* and *Acaulospora* species germinate and grow more successfully in acidic soils than spores of *Glomus* species (Clark et al., 1997; Hepper, 1984; Siqueira et al, 1984; Varma and Hock, 1998). Optimum temperature for spore viability is difficult to determine because it depends not only on the mycorrhizal species, but also on the species

of the plant host. Moisture plays an essential role in spore germination. Without an adequate amount of water, no germination will occur. The optimum moisture proportion for obtaining high germination is also dependent upon species. Mineral content in the soil appears to have less impact on spore germination (Bartolome and Schenck, 1994); inhibition of spore germination in *Glomus* spp. by phosphorous is dependent on rate of phosphorous (Hepper, 1983). Soil microbes also affect spore germination. Bacteria are the most well studied organisms, and their impact is dependent upon genus, species, and isolate. Some species of *Bacillus* stimulate the germination of *Glomus* spp. (Xavier and Germida, 2003).

After spores break dormancy and germinate in soil, plant roots attract the fungal hyphae through a complicated molecular dialogue between the symbionts. Gene expression and the production of the strigolactones are considered to be essential elements in this dialog. Also, signals from the host known as branching factors (BFs) induce gene expression and enhance the growth of the fungal hyphae. Once the hyphae reach the plant surface, they form appressoria, which are the infection apparati of the fungus. The main function of an appressorium is to penetrate the plant epidermis, thereby establishing an easy access for fungal development (Reinhardt, 2007; Lambais, 2006; Harrison, 2005). Appressorial formation occurs exclusively in the host plant, yet the signals that trigger this are unknown. Spores of *Gigaspora margarita* Becker and Hall germinated and formed appressoria followed by strong penetration into the cortical cell of carrot (*Daucus carota* L.), whereas in common beet (*Beta vulgaris* L.), non-host, weak appressorial formation, and undeveloped hyphae were documented; in the latter case, infection was not established (Nagahashi and Douds, 1997). Calcium, calmodulin, and other gene products released from the outer layer of the cell wall of the plant are involved in appressorial initiation (Liu and Kolattukudy, 1999; Breunninger and Requena, 2004; Shaw and Hoch, 2000). After appressorial formation has been established, a specific elaborate channel is established by the plant host cell [the pre-penetration apparatus (PPA)], which serves as a bridge connector between the appressorium and the plant cell lumen (Genre et al., 2005; Genre et al., 2009). Before the entry of the fungal hyphae into the plant cell, plant organelles undergo cytological rearrangements; the nucleus migrates

from a peripheral position to a central position in the plant cell at the site of hyphal penetration. In some cases, the nucleus enlarges. Other cellular organelles such as vacuoles, mitochondria, and plastids also undergo major alterations during formation of arbuscules, the advanced structure of AMF.

Plastids are considered to be important organelles for maintaining and successfully establishing root mycorrhization (Balestrini et al., 1992; Gianinazzi, 1996; Fester et al., 2001; Lohse et al., 2005). In plant roots, plastids play major roles in cellular physiology including the production of fatty acids, amino acids, and apocarotenoids and the assimilation of nitrogen (Fester et al., 2001). Plastids are also involved in carbohydrate metabolism. Plastids have direct effects on AMF symbiosis. Firstly, periarbuscular membrane of AMF consists of fatty acids (Pumplin and Harrison, 2009). Secondly, there are several enzymes (e.g., nitrite reductase, and glutamine synthestase) located in the plastids that regulate nitrogen uptake by the fungus. Lastly, plastids control the availability of the microsymbiont's carbohydrate.

Fungal hyphae enter the cortical cell through the PPA trajectory channel and grow either intracellularly or intercellularly in the apoplast. When hyphae reach the cortex, they start differentiating to form a uniquely distinctive feature known as an arbuscule. The name arbuscule is derived from the Latin word arbusculum, which means a small tree. Arbuscules are described as highly coiled branches of hyphae that occupy the plant cell, and they function as the main site for exchanging of mineral nutrients and carbohydrate between the fungus and the plant. The estimated life span of an arbuscule varies; after a few days, the mature arbuscule begins to collapse, and forms a clump-like structure. Ultimately, the degenerated arbuscles disappear and leave the area for other newly formed arbuscules to re-colonize the plant cell (Alexander, 1988; Harrison, 1999; Hause and Fester, 2005).

When hyphae penetrate the plant cell, the cytoplam invaginates and engulfs the hyphae forming a unique structure called " the periarbuscular membrane (PAM)." The PAM provides an extensive surface area for exchanging nutrients and carbohydrate

between the mycosymbionts and is composed of fungal cell wall and plant cell wall. The PAM consists primarily of two main sections: arbuscular branches and the arbuscular trunk domains. Many phosphate transporter proteins that exist only in mycorrhizal hosts are found on the PAM; these include the *Medicago truncatula* Gaertn. Pi transporter (MtPT4) (Javot et al., 2007) and the rice (*Oryza sativa* L.*)* phosphate transporter gene OsPT11(Paszkowski et al., 2002). In soybean (*Glycine max* L.), the ammonium transporter (*Gm*AMT4.1), visualized using a *Gm*AMT4.1–green fluorescent protein fusion, was found in the PAM but only in the branch domain and not in the trunk region (Kobae et al., 2010). The PAM also contains proteins that generate the ATPase activity needed to energize nutrient exchange.

Vesicles formed by some AMF simultaneously with formation of the arbuscules are found in different positions in the plant cortex, such as intercellular, intracellular or terminal (Smith and Read, 2008). The majority of AMF species produce vesicles; members of two families, Gigasporaceae and Acaulosporaceae do not form vesicles but instead, form auxiliary cells that serve the same function. Vesicles function as storage compartments for lipids and are comprised mostly of lipids (Smith and Gianinazzi-Pearson, 1988; Smith and Read, 2008).

Taxonomy-Phylum Glomeromycota. Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil inhabitants that form symbiotic associations with root systems of most plant species. Schüβler et al (2001) removed AMF from Phylum Zygomycota and re-classified them as Phylum Glomeromycota based on small subunit rRNA gene sequences. This reclassification was supported by further studies with phylogenetic studies with the RNA polymerase II subunit B1 (rpb1) gene (Redecker and Raab, 2006). The phylum contains ten genera in eight families (Redecker and Raab, 2006). Two AMF species used in this study will be discussed further: *Gigaspora margarita* (*Gm*) and *Glomus intraradices* (*Gi*) Schenck and Smith.

Gigaspora margarita *Gigaspora margarita,* like other members of the Family Gigasporacae, does not produce vesicles; however, an auxiliary cell formed

intercellularly is covered with echinulate spines. Mature spore colors are varied from white to yellow, and their size is relatively large. Spores are globose, and several germ tubes can be initiated during the onset of germination (Bentivenga and Morton, 1995). The spore wall is constituted of four layers (Sward, 1981). Both arbuscules and hyphae are observed in this genus. Also, this species is the best example of the *Arum*-type mycorrhizae type, which is distinguished by its intercellular hyphal penetration of the host cell during the AMF colonization.

Glomus intraradices. The genus '*Glomus'* is considerd to be the largest of the AMF (Schwarzott et al., 2001). Mature spore walls contain two zones divided into an outer and inner zone, and each zone is composed of several layers (Maia and Kimbrough, 1994). In contrast to *Gm*, *Gi* represents *Paris*-type mycorrhizae that are characterized by intracellular hyphal penetration of the host cell (Armstrong and Petersson, 2002). Furthermore, *Gi* differs from *Gm* because it forms vesicles.

1.3. Macronutrient Uptake in Mycorrhizal Plants

Phosphorus. Phosphorus (P) is classified as macronutrient, composing almost 0.2% of plant dry weight. It is a pivotal substance in intracellular energy transfer (ATP), nucleic acids, phospholipids, and enzymes. Phosphorus can be found abundantly in the soil in various forms such as amorphous phosphate, polyphosphate, and orthophosphate, but the only form that is accessible to host roots is the orthophosphate (Pi) type (Karandashov and Bucher, 2005; Schachtman et al., 1998). Plants absorb Pi directly from the soil via root hairs that reach into the P zone and translocate it to the plant to be utilized. However, plant consumption rate of P is much greater than Pi availability within the root area; this is known as the depletion zone (Jansa et al, 2011). Symbiotic relationships with AMF increase P uptake; this may have led to high numbers of terrestrial plants being colonized (Smith and Read, 2008). In addition to direct uptake of P by roots, AMF hyphae increase P uptake by extending beyond the range of the root hairs to obtain P and transfer it to the plant; the hyphae can penetrate the small pores of soil particles.

In general, colonization by AMF is lower if there is a high concentration of P, irrespective of its form. At a high rate of P, onset of both entry points and vesicles in leeks (*Allium porrum* L.) is reduced; these are essential for colonization by *G. mosseae* (Amijee et al., 1989). Chile pepper (*Capsicum annum* L.), cilantro (*Coriandrum sativum* L.), tomato (*Solanum lycopersicum* L.), and corn (*Zea mays* L.) plants exposed to high P application all had a significant decreases in AMF colonization (Schroeder and Janos, 2004). Therefore, commercial or indigenous AMF application has the potential to decrease the cost of P on agricultural lands.

Nitrogen. Nitrogen (N) is available to plants in various forms such as nitrite (NO_3), and ammonium (NH_4) ions. Nitrogen is more accessible to plants than P; therefore, little research has been focused on N acquisition by AMF (Javaid, 2009; Jin et al. 2005). Extraradical mycelia (ERM) are believed to be the main means of acquiring N; the ERM extend a few centimeters outside the root zone to reach N sources and translocate N to the plant host (Frey and Schiipp, 1993). Nitrogen taken from the soil via ERM is assimilated into the arginine through various enzymes and reactions such as nitrate reductase, glutamate dehydrogenase, and glutamine synthetase-glutamate synthase (GS-GOGAT). Arginine is the prevalent amino acid component that is transferred from ERM to intraradical mycelium (IRM) at the interface with the plant host. In IRM, arginine is converted into the NH_4 form by ornithine aminotransferase and urease enzymes that are specific to mycorrhizal roots and delivered to the plant host (Govindarajulu et al., 2005). A mycorrhizal gene (LjAMT2; 2) that was upregulated in arbuculated cells of *Lotus japonicus* (Regel) K. Larsen colonized by *G. margarita* transported only the NO_3 form (Guether et al., 2009).

Application of NH_4 as a nitrogen source has detrimental effect on AMF colonization because it results in changes in the rhizosphere [e.g., increased P concentration and reduced pH (which has deleterious impact on spore germination) (Hawkins and George, 2001)]. Use of NH_4 can also result in reduced cell wall permeability and subsequent reduction in root exudates essential for mycorrhizal spore germination (Hawkins and George, 2001).

Potassium. In general, AMF can also improve plant uptake of potassium (K). For instance, onion plants (*Allium cepa* L.) colonized by consortia of *Glomus* species (*G. versiforme*, *G. intraradices*, and *G. etunicatum*) had greater K content in shoots than non-mycorrhizal plants; there were no differences among *Glomus* species (Aliasgharzad et al., 2009). The amelioration of plant K varied by AMF isolate. Cassava plants colonized with *Acaulospora myriocarpa* (Sieverding and Schenck) or *Glomus occultum* (Walker) had higher K and P content than plants colonized by *A. longula*, *Entrophospora colombiana* (Schenck), *G. fasciculatum* (Thaxt.) Gerd. & Trappe, or *G. manihotis* (Sieverding and Toro, 1988). In addition, there is evidence that colonization by AMF enhanced acquisition of K by *Panicum virgatum* L. in acidic soil (Clark et al., 1999). Potassium-induced jasmonic acid reduces insect herbivory and may also impact tolerance to plant pathogens (Amtmann et al., 2008).

1.4. Allelopathy

Allelopathy is "any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influences the growth and development of agriculture and biological systems" (International Allelopathy Society, 1996). The term allelopthy was originally derived from Greek words, *allelon* which means of "each other", and *pathos* which means "to suffer" (Singh et al., 2001). Allelopathy can be negative or positive for agricultural systems. The detrimental impacts include: growth inhibition or reduction of the crop plant, change in the genetic codes of plants (mutation), and inhibition of nutrient uptake by plant roots. Beneficial effects include: manipulating this phenomenon to suppress weeds in field crops, and using allelopthic crops in lieu of pesticide applications in order to kill the weeds in the field.

Chemicals that induce allelopathic responses are known as allelochemicals or allelochemics (Whittaker and Fenny, 1971). Allelochemicals are secondary metabolites that are produced by one plant (donor) and negatively impact another plant (receiver). Allochemicals can directly or indirectly have a negative impact on the receiver plant, and

soil environment. Most allelochemicals are phenolics or terpenoids; these types of compounds exhibit a huge chemical diversity and are engaged in a number of metabolic and ecological processes. Allelochemicals are released from plants into the environment through leaching, volatilization, and root exudations.

1.5. Sorghum - *Sorghum bicolor* (L.) Moench.

Sorghum is a cereal crop used for an array of functions throughout the world. Sorghum is used as a cover crop (especially in the United States), a green manure crop, a main crop in crop rotation, and as livestock fodder. Sorghum is well known as an allelopathic crop and is widely used to suppress weeds. Sorghum exudates in the soil or living roots inhibit or decrease the growth or yields of successive crops. Seedlings of wheat were partially inhibited by the presence of mature sorghum during early germination; however, since there was no substantial yield loss in wheat, the allelopathic effect of sorghum was thought to be degraded in the soil (Roth et al., 2000). Although the introduction of sorghum to crop rotation could result in negative impacts on subsequent crops, it will also suppress the growth of weeds that compete for water, nutrient, light, and space with the desired crops. Conversely, if weeds are more inhibited by sorghum than the original crops, crops will grow or prosper because there would not be competition by weeds. In many cropping systems, rotation with sorghum reduces the incidence of Verticillium wilt; rotation of cotton fields with sorghum for two years reduced *Verticillium* inoculum (Woodward et al., 2010).

The primary allelochemical produced by sorghum roots is sorgoleone (2-hydroxy-5-methox-e- [(82,112)-8,11,14-pentadecatriene]-p-benzoquinone) (Netzly and Butler, 1986). The major negative effects of sorgoleone compounds on subsequent crops are reduction of seed germination and seedling growth, and reduction of chlorophyll (Gniazdowska and Bogatek, 2005). Planting sorghum as a cover crop in order to reduce the density of weed populations in the field could lead to significant decline in the biomass of subsequent crops.

1.6. Wheat - *Triticum aestivum* (L.).

Wheat is one of the most important cereal and staple crops in the world, and domestication of wheat led to the development of agriculture-based human societies. Wheat is classified in the genus *Triticum* (Family:Gramineae); the number of species in the genus varies based on the classification system, but modern classification places the number of species at about 30 (Goncharov, 2011). In 2008-09, world production of wheat was 656 metric tons (Anonymous, 2008), making it the third most grown crop after rice and maize.

The form in which wheat is consumed varies. Some uses are: food (e.g., bread and cookies), livestock feed, fermented beverages, and more recently biofuels. In some countries, especially developing countries, wheat is the most available source of protein; it is hard to determine the percent of protein because it depends on many factors. To address this dilemma, scientists have tried to find a solution by applying AMF; in some areas, these may already exist in the soil. These fungi provide wheat with tremendous benefits, (alleviating mineral nutrient deficiencies, increasing water uptake, and providing protection from pathogens).

1.7. Insect-plant interactions

Herbivorous insects are divided into main types based upon their feeding behaviors: chewing and sucking (phloem feeding). One chewing insect [fall armyworm (*Spodoptera frugiperda*, J. E. Smith)] and one phloem-feeding insect [bird cherry-oat aphid (*Rhopalosiphum padi* L.)] were used in this research.

Fall armyworm (FAW). The FAW (Lepidoptera: Noctuidae) is a chewing insect and a serious economic pest that infests a wide range of plant crops such as wheat, rice, sorghum, maize, cotton (*Gossypium hirsutum* L.) and barley (*Hordeum vulgar* L.) (Alton, 1979; Nagoshi, 2009). This pest has two unique sympatric and morphological strains: the first is known as the corn strain (C-strain), and the second is the rice strain (R-strain) (Nagoshi and Meagher, 2008). The two strains differ in plant preference and insecticide tolerances (Diez and Benjumea, 2011).

Aphids. Aphids are efficient phloem feeders and one of the largest orders (Hemiptera: Aphididae) of insects. Despite their minute size, these insects cause considerable damage to agricultural crops (e.g., wheat, barley, and tomato). They cause harmful effects by consuming plant carbohydrates, producing honeydew (fungi that grow on the honeydew block light absorbtion thus reducing photosynthesis), inducing galls, and transmitting plant viruses (Guerrieri and Digilio, 2008; Smith and Boyko, 2007). Honeydew attracts different kinds of natural enemies of aphids such as parasitic wasps, and also stimulates the growth of saprophytic fungi (e.g., *Septoria nodorum* Berk.) on host leaves (Fokkema et al., 1983).

Several microorganisms are recognized (fungi, nematodes, mites, beetles, whiteflies) as vectors for plant viruses (Ng and Falk, 2006; Powell et al, 2006). Aphids transmit many economically important viruses and are particularly effective for transmitting plant viruses for several reasons:

1. They can quickly colonize a plant host because they reproduce frequently. They have a short life cycle and can switch between two types of reproduction (parthenogenesis or sexual mating) depending upon resource availability and environmental conditions;
2. They have a wide host range (Ng and Perry, 2004; Hodge et al., 2011);
3. They utilize their stylet to penetrate the plant cell tissue to obtain plant sap and ingest the virus along with the carbohydrate. The stylet serves as a delivery method for the virus when the aphid feeds on a new host. They produce two kinds of saliva (gelling and watery saliva) from their stylets. The saliva protects the stylet during penetration by forming a sheath-like structure that suppresses host defense; this allows efficient transfer of the viral particles (Moreno et al., 2011).

Viral transmission by aphid vectors has been classified into two modes: circulative and non-circulative. In the former, viral particles (virions) are taken from the infected plant tissue, transferred through the food canal, foregut, midgut, and hindgut, and retained within the haemocoel; eventually the virion is introduced into a new host via

the aphid's saliva. The circulative mode is further divided into two types: propagative viruses are those that replicate inside the plant host cell [e.g., Lettuce necrotic yellow virus (LNYV)], and non-propagative viruses are those that cannot replicate within the plant cell [e.g., Potato leaf roll virus (PLRV)] (Brault et al., 2010; Ng and Perry, 2004). In contrast to the circulative mode, non-circulative viruses are retained only within the aphid mouthparts (i.e., externally on the stylets) and foregut; and the virus cannot circulate within the rest of aphid body (Ng and Perry, 2004). Also, the non-circulative mode is characterized by a short acquisition period, and the virus does not persist in its vector very long before injecting it into a new host. Viruses transmitted in a non-persistent manner (e.g., Tobacco etch potyvirus) and those transmitted in the semi-persistent manner (e.g., Beet yellows closterovirus) are the main types of non-circulative transmission. Although they lack the ability of movement and circulation within its carrier (i.e., aphid), these viruses are readily transmitted because they are retained on the stylet and the aphid cuticle (Brault et al., 2010). These viruses can be lost during the aphid molt so there is a short retention time (Ng and Falk, 2006).

The life cycles of aphids can be very complex. Aphids generally have an alate life cycle, in which winged adults develop and reproduce sexually, and a parthenogenetic cycle, in which wingless females produce live young rather than laying eggs. Parthenogenetic females produce offspriung rapidly, resulting in large, plant-damaging populations. Aphids can be either autoecious (the aphid completes its life cycle on the same host) or heteroecious (the aphid has two unrelated hosts) (Dixon, 1971). In the heteroecious aphids, the primary host is usually a woody plant, and the secondary host, is often a grass or cereal crop. For example, bird cherry-oat aphid colonizes bird cherry tree (Family: Rosaceae) as the primary host and many different members of the grass family [e.g., oats (*Avena sativa*: Poaceae)] as secondary hosts during the summer season. Aphids are produced in two forms: alate males (i.e., where nymphs possess wings) or apterous females (i.e., where nymphs lack wings). Plant host volatiles and aphid behaviors determine the proportional rate of the forms (Glinwood and Pettersson, 2000). More individuals of the alate type are produced more during aphid aestivation, or if the host is overcrowded. Conversely, more individuals of the apterous type are produced during the

build-up of a new colony (Powell and Hardie, 2001). The life cycle of *R.padi* L. commences with mating between alate and apterous forms on species of *Prunus* in the fall; eggs are laid on the woody host. When the egg hatches, it produces a fundatrix (i.e., the first spring generation), and the fundatrix remains on the tree until they form wings to migrate to the secondary host. Migration to the grass host occurs mostly in the summer, exules (i.e., summer generation) are produced on the grass host, and the life cycle repeats itself again (Lukasik, 2009; Powell and Hardie, 2001).

Mechanisms of host selection and alteration are not well understood, but several hypotheses can be found in the literature. In the first hypothesis, aphids compensate for the decline in nutrition of the primary host by moving to the nutrient-rich secondary host. During the summer, phloem in the leaves of the woody host (e.g., *Prunus*) is reduced and the relative nutrient content (i.e., nitrogen) decreases in the phloem of the herbaceous host; aphids migrate to the woody host in order to exploit a better quality of sap contents (Sandström, 2000). In the second hypothesis, aphids are thought to change hosts to avoid their natural predators. Some predators are conditioned to seek insect hosts based on the plants on which their previous prey fed. For example, in the absence of aphid hosts, females of the parasitoid wasp, *Aphidius colemani* did not preferentially select plants. Wasp response, however, was drastically altered when faced with aphid-infested plants. Wasps emerging from *Myus persicae* (Sulz.) preferred to return to infested plants on which their prey had been reared (Bilu et al., 2006). The third hypothesis is that allelochemicals (phenolic derivatives, chologenic and tannic acids) manufactured by the primary host may act as deterrent components or reduce reproduction (Czerniewics et al., 2011). For example, higher concentrations of methyl salicylate are produced by *Prunus* leaves in the summer than in the winter. Because at high concentrations, methyl salicylate is repellent to *R. padi*, this forces the insects to escape and look for another host (Pettersson et al., 1994). Endophyte infection of tall fescue is well known to alter plant chemistry, feeding preference and survival of herbivorous insects (Ball et al., 2011). In a study on the influence of endophyte infection status of tall fescue (*Lolium arundinacea* Schreb.) on *R. padi* colony sizes, not only was colony size predictably reduced, but production of alate forms in response to a predator was also decreased on endophyte-

infected plants. Although all colonies on endophyte-free grasses produced alate forms, only a few colonies on endophyte-infected plants produced alates. These few colonies, however, were able to produce winged forms on endophyte-infected grasses; however, these few colonies produced larger proportions of winged morphs than alate colonies on endophyte-free grasses. Without a predator threat, no colonies on endophyte-infected grasses produced any winged morphs (Züst et al., 2008).

Aphid Predators. Many predators consume aphids as a major dietary source and have been developed as a control strategy in controlled environments (e.g., greenhouses and nurseries). For example, lacewing larvae (*Dichochrysa prasina* Burmeister), seven-spotted lady beetle (*Coccinella septempunctata* L.), and Asian lady beetle (*Harmonia axyridis* Pallas) are predators of the soybean aphid (*Aphis glycines* Matsumura) (Pappas and Koveos, 2011; Xue et al, 2009). Members of the Carabidae family (*Synuchus nivalis* Panzer, and *Agonum dorsale* (Pont.) are reported to feed upon bird cherry oat aphid (Chiverton, 1987). Two other carabid predators of *R. padi* (*Bembidion lampros* Herbst, and *Pterostichus* spp: Coleoptera) caused an effective reduction in the economic threshold of *R. padi* only if the predators were introduced at onset of aphid landing on the plant host (Ekbom et al., 1992). Spiders (e.g., *Mermessus denticulatus* Banks) also consume *R. padi* (Gavish-Regev et al., 2009). Larvae of the ladybeetle (*Adalia bipunctata* L.), a member of the Coccinellidae family: Coleoptera, was an effective predator of *R. padi* at high temperature (21 ºC), but consumption was slower at low temperature (14 ºC) (McMillan et al., 2007). Another coleopteran, *Hippodamia convergens* Guërin-Mëneville, fed upon *R. padi*, but the predator preferred the greenbug aphid, *Schizaphis graminum* Rond, when the two aphid species were presented to the predator either mixed or alone (Phoofolo et al., 2007). Parasitoid wasps have the propensity to parasitize *R.padi,* and they have been used with limited success as biocontrol agents in field crops because of reduced abundance. *Trioxys sunnysidensis* Fulbright and Pike, n. sp., (Braconidae: Hymenoptera), which was isolated from reed canary grass (*Phalaris arundinacea* L.) attacked, and reduced the level of *R. padi* infestation on potted wheat plants (Fulbright and Pike, 2007). Infestation of barley plants by *R. padi* attracted the model aphid parasitoid *Aphidius colemani* Viereck (Fujinuma et

al., 2010). Females of *A. colemani* laid more eggs, and consequently produced more surviving offspring on hosts that were infested with three aphids [*Aphis gossypii* Glover (cotton), *Myzus persicae* (radish), or *Schizaphis graminum* Rondani (barley) (Homoptera: Aphididae)] than *R. padi* on barley, but *R. padi* influenced the sex allocation ratios of *A. colemani* via stimulating production of females more than the other aphids (Ode et al., 2005). For another parasitoid (*Aphidius rhopalosiphi* De Stefani-Perez), the density of aphids on leaves was important for parasitism. When *R. padi* infestation of wheat was high (9 aphid/cm), *A. rhopalosiphi* was an effective parasitoid, whereas, at the lower density (1 aphid/cm), it was not; this was attributed to the volatile spacing pheromones that were produced only at the high density (Gonzáles, 1999). These experiments on predation of aphids were done under controlled conditions (i.e., greenhouse), and these results may not be reproducible in the field.

Because of the economic losses due to aphid feeding, virus transmission and honeydew, aphid control is important in crop production, but control is problematic. Insecticides that control aphids pose a public health concern since these not only impair the nervous system of the pest, but they can impact humans in the same manner (Dedryver et al., 2010). Also, because of the rapid rate of reproduction, aphids quickly develop resistance to pesticides (Edwards et al., 2008).

Aphid pheromones. Aphids produce different types of pheromones essential for their survival, dispersal, and reproduction. The amount and rate of the pheromones is varied, depending upon the species of aphids, environmental factors, and plant host. The word pheromone is derived from Greek words *pherein*, which means transfer and *hormone*, which means excite (Dewhirst et al., 2010; Nation et al., 2000). Sex, alarm, and aggregation pheromones are the most abundant pheromones released by aphids. Aggregation and sex pheromones are often used interchangeably in the literature, but the term aggregation is used when the pheromone attracts both genders on the same host; whereas, if the pheromone is emanated by the female and attracts males, it is referred to as a sex pheromone (Landolt and Phillips, 1997).

Owing to the complexity and alteration of the aphid life cycle, females (especially females that are produced parthenogenetically on the primary host) arrest wandering males via release of sex pheromones. These pheromones are produced from scent plaques in their rear tibiae. Males detect the sex attractants through specific olfactory receptors located on their antennae, and the pheromones act as aphrodisiac stimulants (Birkett and Pickett, 2003). Many pheromones have been studied and identified by using gas chromatography (GC) coupled with mass spectrometry (MS) methods. For example, the monoterpenoids including (4aS, 7S, 7aR)-nepetalactone and (lR, 4aS, 7S, 7aR)-nepetalactol, are predominate components in pheromones produced by *Aphis spiraecola* Patch, *R. padi,* and *Phorodon humuli* (Schrank) (Jeon et al., 2003; Pope et al., 2007). Plant host chemistry can alter chemical and biological properties of sex pheromones (Landolt and Phillips, 1997). A mixture of two volatile compounds, (benzaldehyde and methyl salicylate) isolated from the *Prunus* host, with the sex pheromone obtained from *R. padi* resulted in synergistic effects; numbers of *R. padi* males caught in traps baited with the mixtures were greater than in those treated with the sex pheromone alone. The combination of the two volatiles and the sex pheromones resulted in a decrease in the numbers of damson-hop aphid *Phorodon humuli* caught compared to the sex pheromone alone (Pope et al., 2007).

Alarm pheromones, the second largest group after sex pheromones, are produced when aphids are attacked or disturbed. The alarm pheromone is secreted by siphunculi (cornicles), which are situated at the bottom of the abdomen of the aphid. They affect several aphid behaviors including jumping, warning neighboring colonies, or falling down from the feeding site of the host. These pheromones can also act to deter predators (Bowers et al., 1972; Dewhirst et al., 2010). The most common alarm pheromone that has been identified is (*E*)-β-farnesene (EBF), which is secreted by the pea aphid (*Acyrthosiphon pisum*) in the presence of its predator lacewing larva (*Chrysoperla carnea*: Neuroptera) (Schwartzberg et al., 2008). The aforementioned pheromone was also produced by *R. padi* cornicles, when the insect was irritated (Wientjens et al., 1973). Similarly, the green peach aphid (*Myzus persicae*) secretes the same EBF volatile (De Vos et al., 2010).

Some species of autoceoius aphids such as *R. padi* have shown the ability to release pheromones known as aggregation, and spacing pheromones, although the volatile components have not yet been identified (Dewhirst et al., 2010). Spacing pheromones are produced when the population number of insects is increasing on their host plants. These pheromones, including, 6-methyl-5-hepten-2-one (sulcatone), (+)-6-methyl-5-hepten-2-ol (sulcatol), and 2-tridecanone, were isolated from wheat plant seedlings that were infested by *R. padi*. . They deterred colonization by aphids of the same species (Quiroz et al., 1997).

1.8. Alterations in host physiology by AMF Colonization

Alterations in host chemistry. Colonization of a plant host by AMF induces many changes not only in root architecture, but also in levels of gene expression. Changes in host chemistry are dependent upon both the host and the AMF species. Barley plant roots that were colonized by *G. intraradices* showed a 4-fold significant up-regulation of jasmonic acid (JA), and its amino acid conjugate JA-isoleucine (JA-Ile) expressions, compared to nonmycorrhizal plants (Hause et al., 2002). Other enzymes [e.g., allene oxide synthase, and jasmonate-induced protein (JIP23)] were also stimulated; increases were detected primarily during the peak colonization of AMF when mycorrhizal infection was high (60%), approximately 8 weeks. In another study on tomato, levels of neither JA nor other related hormones were affected by colonization with either *G. intraradices* or *G. mosseae*. However, salicylic acid (SA) levels were slightly increased in *G. mosseae* roots than in *G. intraradices* roots. Surprisingly, ethylene (ET) expression was reduced in both AMF roots (Lopez-Raez et al., 2010). Bean plants colonized by *G. mosseae* showed neither decreased nor increased plant defense-related genes such as chitinase, B-1, 3-glucanase, and phenylalanine ammonialyase (PAL) (Mohr et al., 1998). However, when the same host was inoculated with *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli*, enzymes were upregulated (e.g., a 3-fold in chitinase; 4-fold in PAL) compared to the control plant.

Protection against insects. Colonization of plants by AMF has been speculated to have a positive effect on specialist insects such as aphids but an opposite effect on generalist insects such as beetles and fall armyworms (Hartley and Gange, 2009). Impacts of AMF colonization on some aphid populations are negative, and others are neutral. Feeding damaged by FAW on crops can be partially mitigated through mycorrhizal applications. Detached leaves of soybean plants inoculated with *Glomus fasciculatum* increased host resistance to both FAW, and corn earworm (*Heliothts zea*. Boddie)(Rabin and Pacovsky, 1985). Larval biomass of both pests in mycorrhizal plants was approximately 40% less than in controls. Moreover, pupal weight of both species was higher for insects fed control leaves than those fed AMF-colonized plants. The authors speculated that mycorrhizae either increased host nutrition or altered plant physiology to produce anti-feedant compounds. Colonization by *G. mosseae* and *G. fasciculatum* protected strawberry from the root-feeding black vine weevil (*Otiorhynchus sulcatus* Fabricius) when they were inoculated with one species of AMF only, but plants colonized with both species were not protected (Gange et al., 2000). The author reasoned that protection of the host by AMF was the result of the induction of anti-herbivore chemical compounds such as phenolics and terpenoids, but lack of protection by dual infection was not explained. Mycorrhization of pea (*Pisum sativum*) by *G. intraradices* increased host resistance against adult weevils (*Sitona lineatus*) (Wamberg et al., 2003). Resistance to this foliage feeding insect was attributed to transference of carbohydrate from the leaf to the root to meet the fungal demand. Also, plants infected by *S. lineatus* had increased mycorrhization as measured by direct count of arbuscules, vesicles, and hyphae at the beginning, but overall colonization level was decreased due to the damage induced by the beetle feeding on the root. Mycorrhizal soybean plants (*Glycine max*) colonized by *G. etunicatum* had more beetles (*Epilachna varivestis*) than nonmycorrhizal plants. The larger and healthier leaves common to mycorrhizal plants may have resulted in colonized plants being the more preferred hosts for a folivorous insect.

The ability of AMF-infected plants to support more aphids may be due simply to the increased vigor of mycorrhizal plants. *Plantago lanceolata* colonized by *G.intraradices* and infested with aphids (*Myzus ascalonicus* and *M. persicae*) supported

larger numbers of aphids than non-mycorrhizal plants; aphid weight and fecundity were also greater (Gange et al., 1999). Timothy (*Phleum pratense* L.) colonized with *G.intraradices* or *G. mossae* generally had decreased aphid population growth (47%) and plant biomass was enhanced (5%). When alfalfa plants were infected with *G. intraradices*, the rate of aphid parasitism by *Aphidius rophalosiphi* (DeStefani-Perez) increased by 140% relative to the parasitism of *G. mosseae*-colonized or control plants. Parasitoid developmental time decreased by 4.3% and weight at eclosion increased by 23.8% on AMF-colonized plants (Hempel et al., 2009). Variation and concentration of phenolics may play an important role in migration of *R. padi* (Czerniewicz et al., 2011). Chlorogenic acid, a phenolic that is high in bird cherry (*Prunus sp.*) leaves when the aphid migrates, can be elevated in the leaves of plants colonized by *G. intraradices* (Ceccarelli et al., 2010).

Protection against pathogens. Colonization by AM fungi can enhance plant host resistance to soilborne plant pathogens by: 1) producing a more robust plant and facilitating availability of nutrients to the host; 2) competing for both space and photosynthetic products with the pathogen; 3) interacting with other rhizosphere microorganisms such as plant-growth-promoting rhizobacteria (PGPR) that are antagonistic to the pathogen; 4) compensating for the damaged tissues; and 5) inducing plant disease resistance genes (e.g., pathogenesis-related (PR) proteins) (Azcón-Aguilar and Barea, 1997; Lioussanne, 2010). Synergism among AMF species has been documented in which one individual has less effect than a consortium of isolates. For example, cucumber plants (*Cucumis sativus* L.) infected with *Fusarium oxysporum* f. sp *cucumerinum* and colonized by *Glomus caledonium* were smaller and had fewer fruit than cucumber inoculated with combinations of *Glomus* spp. and *Acaulospora* spp. (Hu et al., 2010).

Bipolaris sorokiniana (Sacc.) Schoem [(Syn: *Helminthosporium sativum* King & Bakke) [teleomorph: *Cochliobolus sativus* (Sacc. in Sorok.)] causes foliar damage on wheat leaves and stems (Matusinsky et al., 2010; Kumar et al., 2007) as well as seedling blight, black point, crown rot, common root rot, and spot blotch (Morejon et al., 2006;

Al-Sadi and Deadman 2010). Spores and hyphae of *B. sorokiniana* excrete prehelminthosporol, a type of toxin that disrupts cell membrane function and consequently leads to necrosis (Nilsson et al., 1993).

In a study conducted on 37 crops that belong to two plant families, Graminaceae and Fabaceae, disease caused by *B. sorokiniana* was reduced in crops inoculated with *Glomus mosseae* (Thompson and Wildermuth, 1989). Preinoculation of barley (*Hordeum vulgare*) seeds with *G. intraradices* reduced the transmission of *B. sorokiniana* from the seeds to aboveground parts including stems. Also, there was no correlation between the degree of AMF colonization, and its effectiveness on the suppression of *B. sorokiniana* on barley (Sjöberg et al., 2007). Colonization of plant host by AMF does not always result in protection against plant diseases. Barley roots that were successfully colonized by a species of *Glomus* were not protected from root-rot caused by *B. sorokiniana* (Wani et al., 1991).

1.9. Research goals

The overall aims of this project were to determine if colonization by arbuscular mycorrhizal fungi (AMF) alters responses of common wheat (*Triticum aestivum*) to stresses caused by allelopathy and biotic agents. The specific goals for this research are to deterimine if: 1) mycorrhizal infection of wheat mitigates allelopathic effect of sorghum on wheat; 2) mycorrhizal plants attract fewer aphids than nonmycorrhizal plants; 3) mycorrhizal colonization of wheat reduces feeding of *Spodoptera frugiperda*; and 4) mycorrhizae increase tolerance of wheat seedlings to *Bipolaris sorokiniana* inoculation.

Chapter 2

Material and Methods

2.1. Mycorrhizal (AMF) treatments

All treatments except control were cultured on *Sorghum bicolor* 'Dekalb DK39Y' unless otherwise noted. The following treatments were used throughout this study:

- C - control (wheat only no sorghum)
- NM - nonmycorrhizal sorghum
- *Gi* - sorghum colonized by *Glomus intraradices* (INVAM# UT118)
- *Gm* - sorghum colonized by *Gigaspora margarita* (INVAM # NC175)

2.2. Chemical materials

Unless indicated otherwise, all chemicals used throughout this study were purchased from Fisher Scientific (Pittsburgh, PA).

2.3. Plant materials and growth conditions

Sorghum seeds were surface sterilized with 3.5% sodium hypochlorite (bleach) for 30 minutes, and washed thoroughly with sterile distilled water three times. A germination test was carried out to confirm that there was no pathogen infection. Inocula were obtained from sorghum pot cultures generously provided by Robert Augé, Plant Science Department, The University of Tennessee, Knoxville, TN. Pot cultures were sorghum grown in Turface® Pro League (Profile Products, Buffalo Grove, IL), an artificial growing medium that was infested with or without AMF. In order to increase inocula for this study, sorghum was grown in Turface® for 3 months in South Greenhouse, The University of Tennessee, with or without AMF. Sorghum plants were fertilized two times a week with macronutrients (nitrogen-potassium- phosphate (N-P-K) ratio of 15-0-15] (JR Peters, Allentown, PA). Mycorrhizal plants received a low dose of potassium (0.6 mM monobasic potassium phosphate), while non-mycorrhizal plants received a high dose of potassium (1.2 mM monobasic potassium phosphate).

Micronutrient was applied monthly (Minor Elements, Hendersonville, NC). Plants were treated with insecticidal soap (Neudorf, Oriskany, NY), as needed. Plants were watered with filter-sterilized deionized water; all watering was done by hand to reduce the risk of cross-contamination among the treatments on the bench. To increase the intensity and quality of light, artificial light (P.L. Light Systems Inc, Ontario, Canada) was provided during the winter. Pot cultures were grown at least 12 weeks to ensure colonization.

For experimental treatments, aboveground portions of sorghum were excised, and wheat seeds 'Pioneer 26R22' were sown. Controls contained neither sorghum nor mycorrhizae.

2.4. AMF inoculation and assessment

The source of AMF inocula was chopped sorghum roots that were previously colonized with either *Gi* or *Gm*. Plastic square pots were covered with a silver gray fiberglass screen (Phifer Company, Tuscaloosa, AL) fitted at the bottom to prevent the substrate from leaking. The AMF inocula were placed between two layers of the media: one at the bottom and the second at the top in order to avoid possible contamination among the treatments.

Sorghum and wheat roots were checked routinely to determine the presence of mycorrhizal colonization as described by Phillips and Hayman (1970). A small portion (100 g) of the root cleansed with tap water to remove soil particles was transferred to a plastic cassette. The cassette transferred to a beaker containing 10% of potassium hydroxide (KOH, 85%) was boiled to clarify the roots for approximately 10 min. After discarding KOH, hydrochloric acid (HCL) (2% v/v) was added to the cassette for 1.5 h. The cassettes were rinsed with sterile deionized water (SDW) three times. Trypan blue 0.05% (wt/v) (MP Biomedicals, LCC, Solon, OH) was used for one hour, and rinsed with SDW two times. Lactoglycerol solution (equal parts of lactic acid, glycerol, and water), was added in order to destain. The cassettes were destained at 4 °C for a week to have better visualization of AMF structures.

Roots were transferred from cassettes, cut into small fragments, mounted on glass microscope slides (25× 75× 1mm), and covered with glass slip (24× 60 mm). Afterwards, AMF structures, such as hyphae, arbuscules, and vesicles were examined. One hundred root counts were performed using a lab counter under the microscope (20 x) according to the gridline intersection method described by McGonigle et al. (1990).

2.5. The effect of mycorrhizae on allelopathy

Experimental Design. Treatments [control, NM, *Gi*, and *Gm* (see Section 2.1)] were replicated eight times. The experimental unit was a pot, and twenty wheat seeds were sown into each pot. The experiment was repeated. Treatments were arranged in a Randomized Complete Block (RCB) design. In separate experiments, mycorrhizal pot cultures were produced on an high-sorgoleone sorghum-Sudangrass hybrid (SX-17) (Advanta, Hereford, TX). Significance levels were set *a priori* at $P = 0.05$ for all allelopathy experiments.

Shoot growth. Plant shoots and roots were separated at harvest, and fresh shoot weight (g) was determined immediately after the harvest. Plant height (cm) was measured from the soil line to the end of the longest leaf blade. Stem diameter (mm) was measured equidistant from the soil line (crown area) to the first leaf using a digital electronic caliper (Marathon Watch Company Ltd, Ontario, Canada).

Root weights. Fresh root weight was determined. Root colonization rate was determined on a subsample (100 g) of roots. The remaining roots were dried in a laboratory oven at 70°C for 7 days.

Chlorophyll determination. Wheat leaf chlorophyll content was determined as described by Porra et al. (1989). Wheat leaves (0.05 g) were grounded in cold methanol (4 mL) using a pestle and mortar. The extract (1 mL) was transferred to a microcentrifuge tube (1.5 mL, Eppendorf Company, Hauppauge, NY), and centrifuged at 500 g for 10 min. Supernatant (1 mL) was transferred to a disposable cuvette (12.5× 12.5× 45 mm) (GMBH, Wertheim, Germany), and absorbance spectrum (A^{670} to A^{640}) was determined

(Shimadzu UV-1601 UV-Vis spectrophotometer, Canby, OR). Chlorophyll a (Chl a), and chlorophyll b (Chl b) concentrations were calculated:

$$\text{Chl a} = 16.29\ A^{665.2} - 8.54\ A^{652.0}$$

$$\text{Chl b} = 30.66\ A^{652.0} - 13.58\ A^{665.2}$$

$$\text{Total Chl} = 22.12\ A^{652.0} + 2.71\ A^{665.2}$$

$$\text{Ratio} = \text{Chl a/ Chl b}$$

Percentage survival. The number of plants was counted every two weeks.

Statistical analysis. The significance of treatment effects on wheat plants was assessed by analysis of variance (ANOVA) (PC-SAS ver. 9.2.3., SAS Institute, Cary, NC), and means were compared with Fisher's Least Significance Difference (LSD) test at $\alpha = 0.05$. The first factor was mycorrhiza, and the second factor was allelopathic effect of sorghum.

2.6. The effect of mycorrhizae on aphid attraction

No-choice experiments. Treatments were NM, *Gi*, and *Gm* (see Section 2.1). The experimental unit was the pot; ten seeds were planted per pot. Three weeks after planting, 20 apterous forms of the aphid were transferred to each wheat seedling using a fine bristle paintbrush. Infested plants were transferred to an insect cage (Bug Dorm Rearing Cage, Rancho Dominguez, CA); each cage had three pots of one of each treatment (NM, *Gi* or *Gm*). There were three cages. After five days, aphids on each plant were counted. Plant survival, shoot height, and shoot fresh weight were determined (as described in Section 2.2). The experiment was repeated. Significance levels of $P = 0.1$ were used in all insect experiments.

Choice experiments. Treatments used in this experiment were: NM, *Gi*, and *Gm* (see Section 2.1), and plants were grown as described for the no-choice test with the exception that in some cages, plants were grown in 150 mL glass jelly jars (Ball, Broomfield, CO) with three holes drilled for drainage. Glass jars were used because volatiles produced by plastic pots interfere with GC-MS analysis. After three weeks,

plants were transferred to insect cages.

Each cage contained either pots or jars. Four pots of plants (one pot of each treatment and a source plant heavily infested with *R. padi*) were grown in each cage; five cages were used. Each treatment plant was placed equidistant from the source plant. The experiment was conducted in the greenhouse. After five days, aphids on each plant were counted. In the initial data analysis, there were no differences among plants grown in pots or jars, so data were pooled for analysis. One jar from each treatment was used for volatile analysis. The experiment was replicated three times. The analysis was performed by Dr. Xinwang Wang in the laboratory of Dr. Feng Chen (Plant Science Department, The University of Tennessee, Knoxville) as described by Yuan et al. (2008).

***Data analysis*.** Data from non-choice and choice were analyzed for significance with Proc Mixed. Significance of treatments was analyzed with F protected LSD of least square means (PC-SAS ver. 9.2.3., SAS Institute, Cary, NC).

2.7. The effect of mycorrhizae on fall armyworm (FAW) feeding

Spodoptera frugiperda cultures. Eggs of *Spodoptera frugiperda* (J. E Smith) were purchased from Benzon Research Inc, (Carlisle, PA). Eggs were shipped in cold insulated plastic bags and were incubated at 25 °C for 3 to 4 days. After eclosion, neonates were transferred via a small paint brush to synthetic wheat germ diet for 24 h and incubated at 4 °C (Wilkinson et al. 1972). Dr. Juan Luis Jurat-Fuentes, The University of Tennessee, Knoxville, generously provided the insect artificial diet. At the termination of all experiments, survival larvae numbers were recoreded for further analysis. Foliar damage caused by *S. frugiperda* was assessed in two ways. In the first method, two researchers developed a visual estimate of the amount of damage based on a scale used to evaluate concrete (The U.S Department of Transportation). Leaves were photographed, and image analysis software (Assess 2.2 Image Analysis Software for Plant Disease Quantification; American Phytopathological Society, St. Paul, MN) was used to estimate percentage consumption. These values were converted to the damage scale used to evaluate FAW feeding on grasses [0 -3 scale in which 0 = no damage and 3 = > 70% of the leaf consumed (Hardy et al. 1985)].

No-choice Experiments. The purpose of this test was to evaluate the effect of mycorrhizae on larval feeding. Treatments (C, NM, *Gm*, and *Gi)* were replicated eight times. The experimental unit was the Petri dish. The experiment was repeated.

Three fresh wheat leaves (ca. 3 cm) from the same treatment were taped at each end to the bottom of a Petri dish (11.8-cm-diameter). The lid of the dish was fitted with a filter paper disc moistened with deionized water; filter paper was used to create high humidity. Each Petri dish contained each treatment (C, NM, *Gi*, and *Gm*). Twenty-five larvae were placed in the center of each dish, and the dish was sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL) to prevent insect escape. Petri dishes were transferred to a dark room (21°C) since the FAW is nocturnal. The experiment was terminated when 50% of foliage was consumed in controls (Crawford et al., 2010).

Choice Experiment - All treatments. Treatments were the same as for the no-

choice experiments (Section 2. 4. 3), except that each Petri dish had a leaf segment from each treatment (C, NM, *Gm*, and *Gi*). There were ten replicate Petri dishes in a completely randomized design (CRD). The experiment was repeated twice.

Choice Experiment - Pairwise comparison. Treatments were the same for the no-choice experiments (Section 2. 4. 3), except that each Petri dish had two leaves from two treatments. Each treatment combination was replicated twice.

Fall armyworm variables. After 5 days, living larvae were counted on each Petri dish for all experiments. Leaf damage and leaf consumed were estimated as described above.

2.8. The effect of mycorrhizae on seedling disease caused by *Bipolaris sorokiniana*

Experimental Design. Treatments were C, Nm, *Gi,* and *Gm*. The experiment was a 2 × 4 factorial (pathogen × mycorrhizae) in an RCB design. Treatments were replicated seven times, and the experiment was repeated. Significant levels of $P = 0.05$ were selected *a priori*.

Bipolaris sorokiniana culture. Two isolates of *B. sorokiniana* (WT65 and CoAlmo 8) previously isolated from switchgrass (Vu, 2011) were supplied by Dr. Bonnie Ownley, Department of Entomology and Plant Pathology, The University of Tennessee, Knoxville. Two culture methods were implemented to obtain pathogen spores. In the first method, cultures were grown on potato dextrose agar (PDA) (Difco, Sparks, MD). A small mycelial plug was placed in the center of the Petri dish (100 × 15 mm) that contained PDA. Cultures were incubated for over two weeks at 25 °C in a growth chamber with a 12-h photoperiod. Sterile deionized water (5 mL) containing Tween-20 (0.01% v/v) was added to the plate, and spores were released by scraping gently with a rubber policemen (Pratt, 2006). In the second method, a small mycelial plug was placed on leaf sections of surface-sterilized greenhouse-grown ‘Alamo’ switchgrass (*Panicum virgatum* L.). Leaf segments (3-cm-long) were submerged in 95% alcohol for 30 s, then transferred to 10% commercial Clorox for 1 min, 95% alcohol for 30 s, and then dried in

a biosafety cabinet. Glass Petri dishes containing three layers of moistened filter paper (90 mm) were autoclaved, and three surface-sterilized switchgrass leaf sections (ca. 3 cm) were placed in each dish. Two mycelial plugs from a culture grown on PDA were placed underneath each leaf; the glass was sealed with Parafilm. Spores were released as described above. A hemacytometer (AO America Optical, Buffalo, NY) was used to determine spore concentrations in the suspension. The suspension was transferred to an aerosol spray bottle (180 mL), and plants were sprayed until wet. Control plants were treated with sterile deionized water. Plants were covered with plastic bags for one week to retain humidity and maintained in a growth chamber [25 °C; photoperiod of 12:12 (L: D)] (Percival, Peny, IA).

Seedling disease rating scale. Foliar wheat seedlings were rated 1 to 6 on a scale designed to encompass general robustness, tillering, extent of lesion development and stunting (Table 2.1).

Table 2.1. Rating scale used to evaluate wheat seedling disease caused by *Bipolaris sorokiniana*. Each rating was a consensus between two evaluators.

Rating	Robust	Tillering	Dead leaves (%)	Lesions (%)	Coalesing (%)	Tip burn	Stunting
1	+	All	< 1%	< 1%	-	-	-
2	+	Few not tellering	< 1%	< 5%	< 5%	+	-
3	-	Few tillering	< 10%	≤ 10%	< 5%	+	+
4	-	No tillering	< 15%	10-25%	<10%	+	++
5	-	No tillering	< 25%	10-25%	< 10%	+	+++
6	-	No tillering	< 50%	< 25%	< 25%	+	++++

Chapter 3

Results

3.1. The effect of mycorrhizae on allelopathy (*Sorghum bicolor*)

No plants that were NM or Control were colonized by mycorrhizae. Colonization level of wheat roots by AMF fungi are shown in Table 3.1. Colonization of wheat seedling roots was greater in Trial A than Trial B. Furthermore, *Gm*-colonized plants were greater than *Gi*-colonized wheat plants.

Table 3.1. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings in sorghum allelopathy trials. Treatments are inocula obtained from either sorghum plants colonized with *Gigaspora margarita* (*Gm*) or sorghum colonized with *Glomus intraradices* (*Gi*).

Treatment	Trial	AC (%)	VC (%)	HC (%)
Gm	A	21	.	80
Gi	A	46	5	63
Gm	B	3	.	20
Gi	B	2	9	10

Summary of statistical values (F-values; *P*-values and degrees of freedom) for all trials can be found in the Appendix 1 (Table A.1).

Control plants (which contained no allelopathic sorghum) in both trials had greater shoot weight than other treatments. No difference in shoot weight was found between mycorrhizal and (NM) non-mycorrhizal plants (Fig. 3.1). Control plants (no-sorghum, no-mycorrhizae) had significantly greater shoot fresh weight than all treatments following sorghum so control plants were removed from the analysis in order to further examine the role of mycorrhizae in the alleviation of allelopathy. There were no differences in plant shoot weight among treatments following sorghum in either Trial A or Trial B (Fig. 3.2). Stem diameter was greater in Control (no-sorghum, no-mycorrhizae) than in other treatments ($P = 0.0009$) in Trial A (Fig. 3.3). Stem diameter

was not determined in Trial B. When the Control plants were removed from the analysis, no differences were found between mycorrhizal and NM treatments in Trial A (P = 0.0905) (Fig. 3.4).

Fresh root weight of wheat in the Control treatment (no-sorghum) was greater than in all other treatments in Trial A. Plants colonized by *G. margarita* had greater fresh root weight than wheat colonized by *Glomus intraradices* in Trial A, but there were no differences in Trial B (Fig. 3.5). Control plants were removed from the analysis to determine if mycorrhiza could alleviate the allelopathic effect of sorghum. Fresh root weight of wheat colonized by *G. margarita* was greater than weight of wheat colonized by *Glomus intraradices* or wheat with no mycorrhizae in Trial A, but there were no differences in Trial B (Fig. 3.6).

Dry root weight was greater in control plants than in all other treatments (Fig.3.7), but the mycorrhizal (*Gi* and *Gm*) and non-mycorrhizal (NM) treatments were not significantly different from one another ($P \leq 0.0001$) in either trial. When the no-sorghum treatment was removed from the analysis, dry weight of wheat roots colonized with *Gm* was greater than dry weights of non-mycorrizal and *Gi* roots in Trial A (Fig. 3.8). No difference was found among the treatments in Trial B (Fig. 3.8).

Chlorophyl A concentration of *Gm*-colonized plants was less than that of *Gi*-colonized plants but neither was different from control or NM. Chlorophyll B content was greater in *Gi*-colonized plants than all other treatments (Fig. 3.9). Chlorophyll concentrations were not determined for Trial B. Total chlorophyll concentration of wheat leaves was less in *Gm*-colonized plants than in either *Gi*-colonized plants or controls (Fig. 3.10).

Control and non-mycorrhizal plants had higher chlorophyll content ratios than either mycorrhizal treatment (Fig. 3.11).

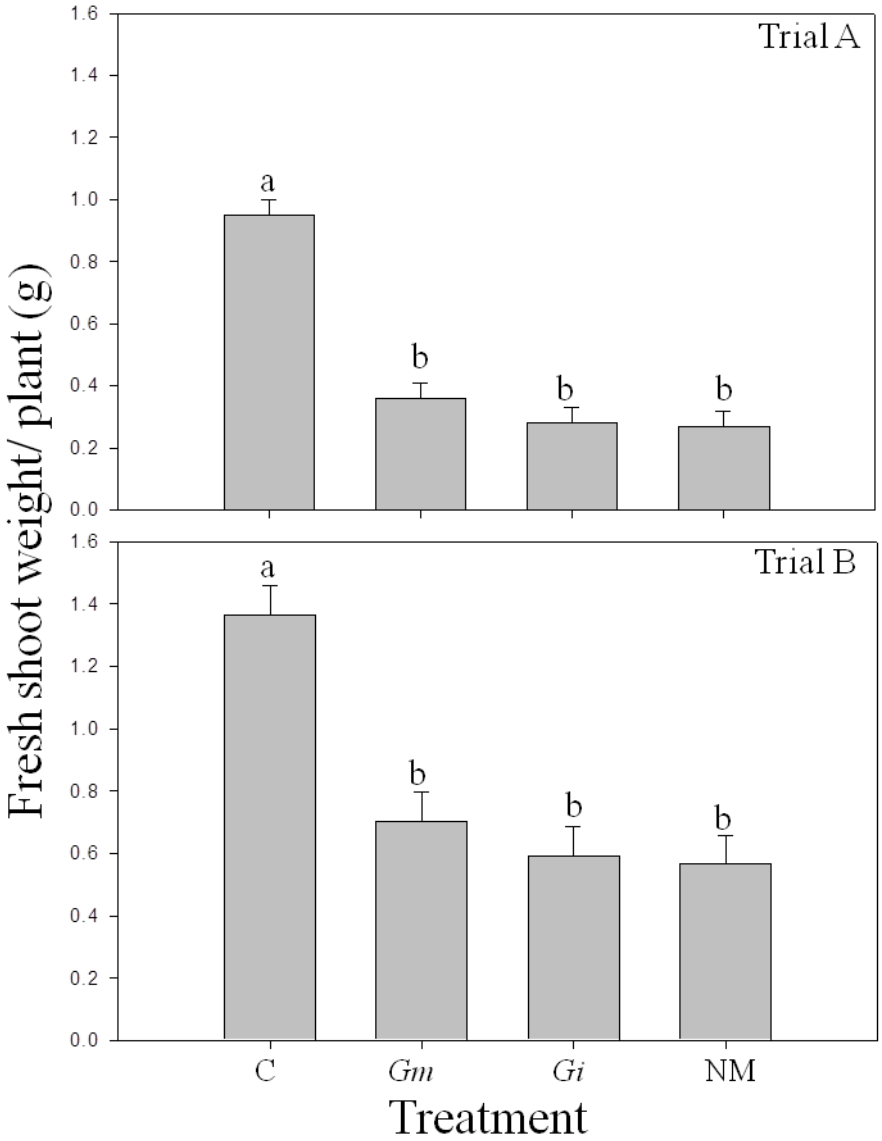

Fig. 3.1. Effect of mycorrhizae on fresh shoot weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter are not different according to an F- protected LSD (*P*= 0.0001 for both trials).

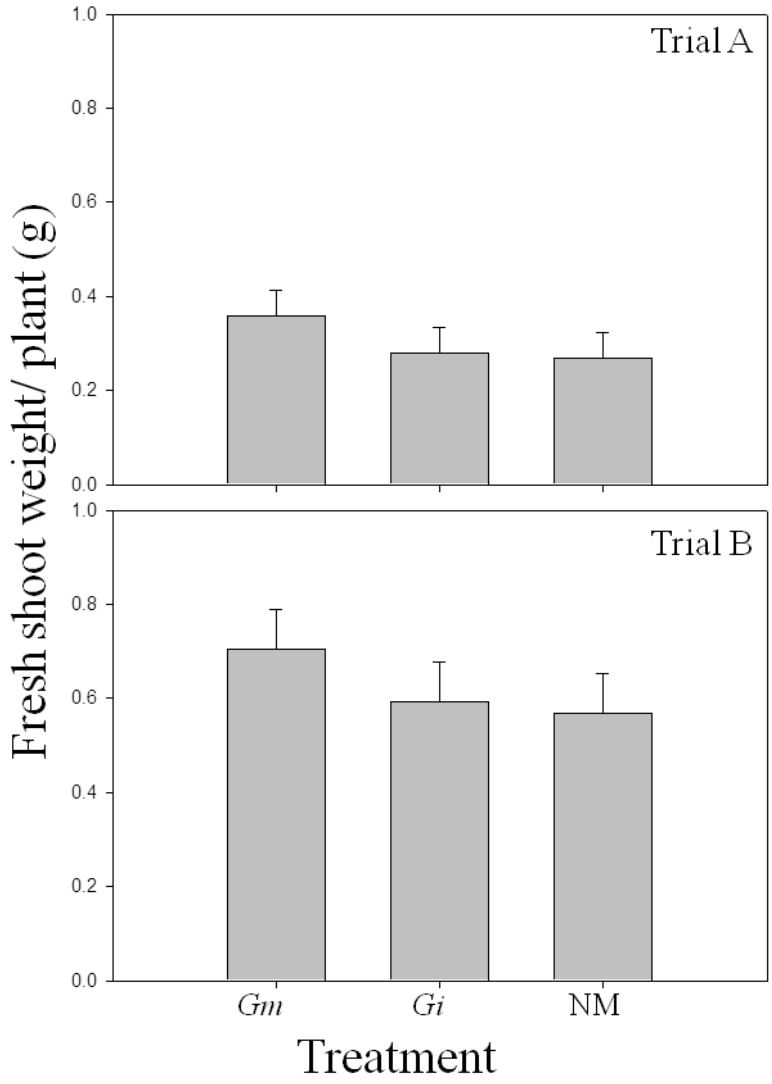

Fig. 3.2. Effect of mycorrhizae on fresh shoot weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD [P= 0.4018, Trial A; P = 0.5008, Trial B).

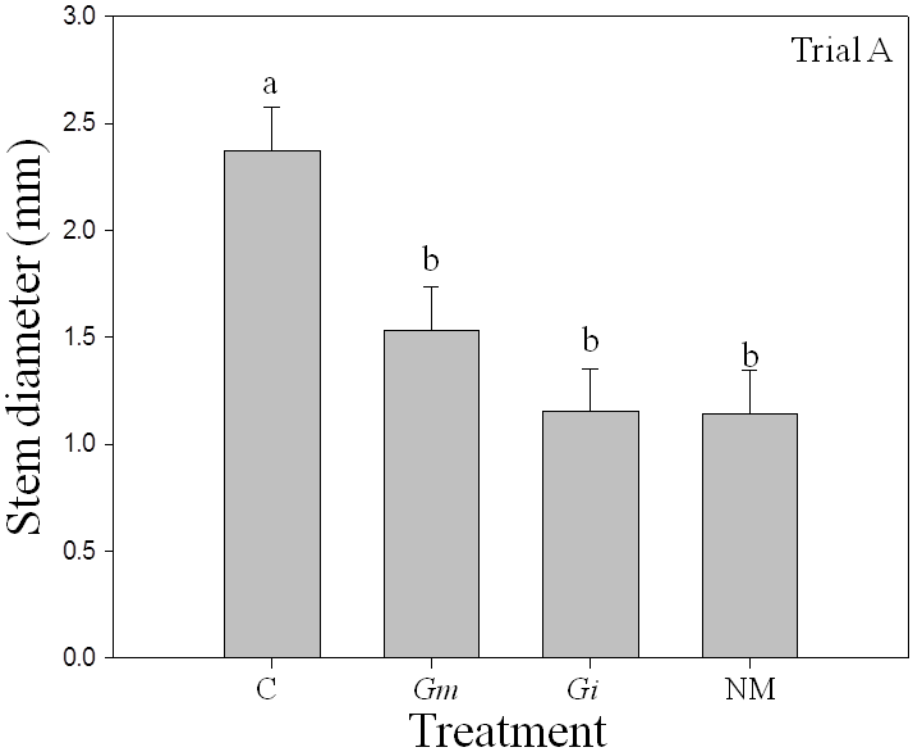

Fig. 3.3. Effect of mycorrhizae on stem diameter (mm) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.0009).

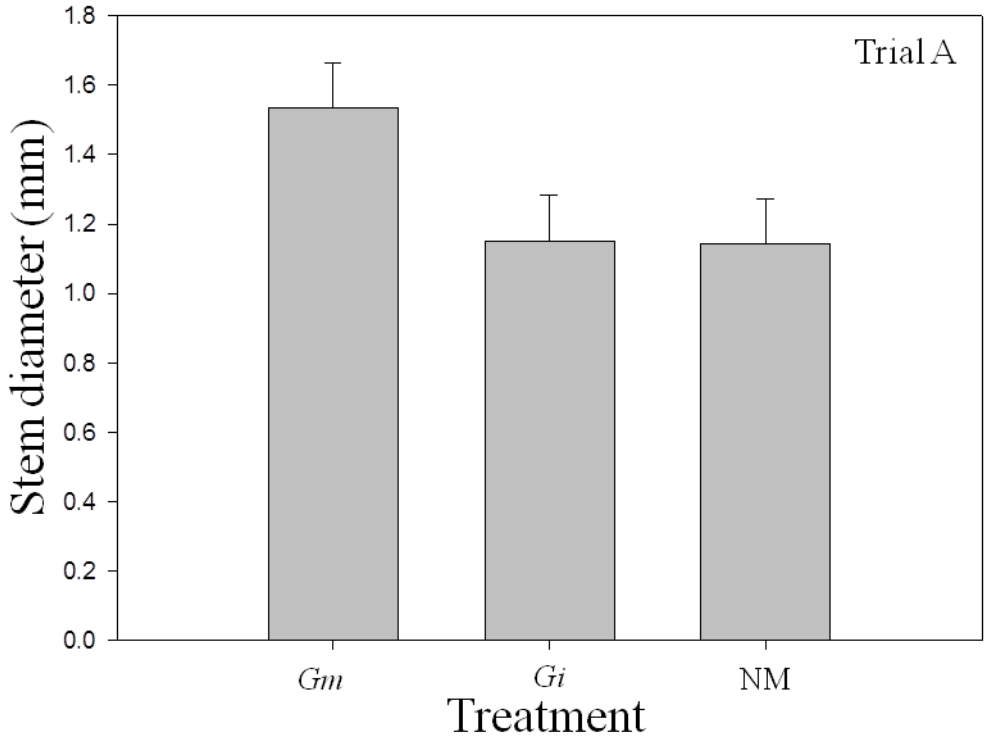

Fig. 3.4. Effect of mycorrhizae on stem diameter (mm) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Bars without letters are not different according to an F-protected LSD (P = 0.0905).

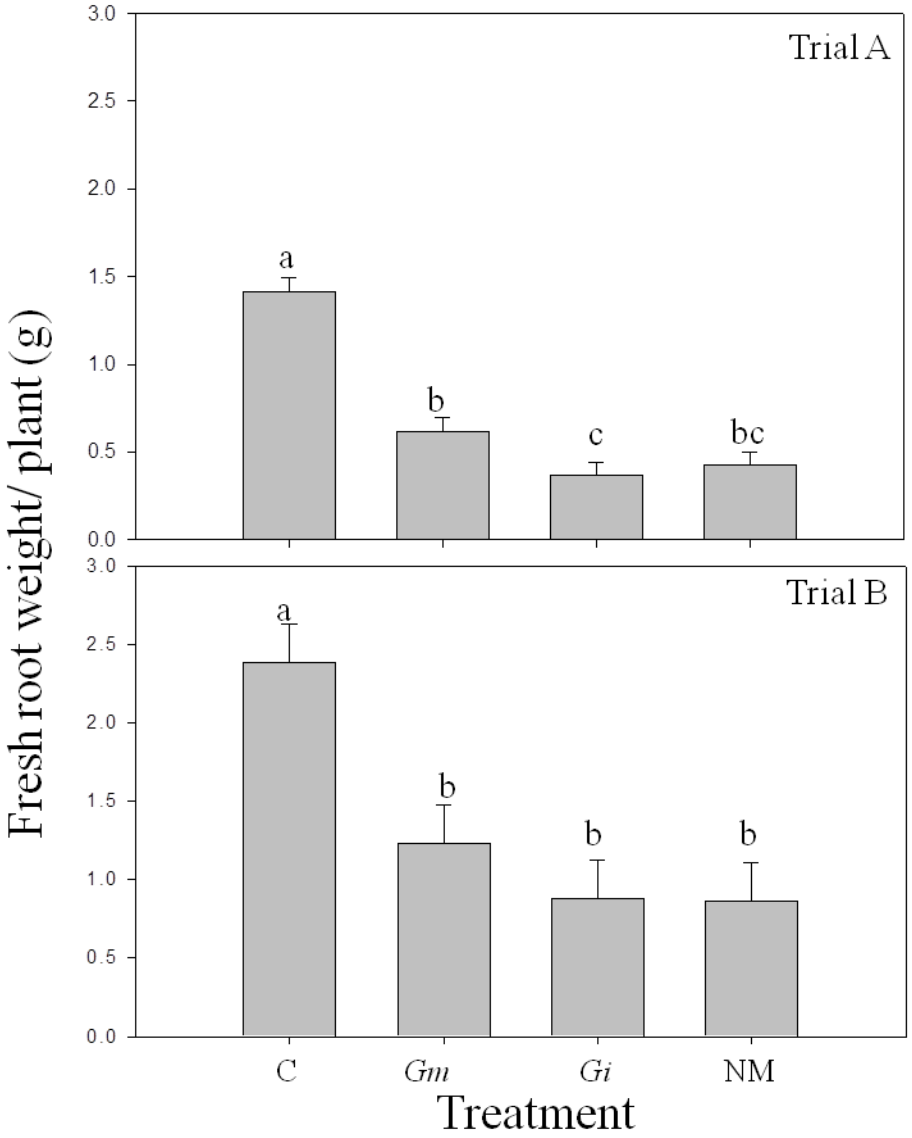

Fig. 3.5. Effect of mycorrhizae on fresh root weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter are not different according to an F-protected LSD (*P*= 0.0001 for both trials).

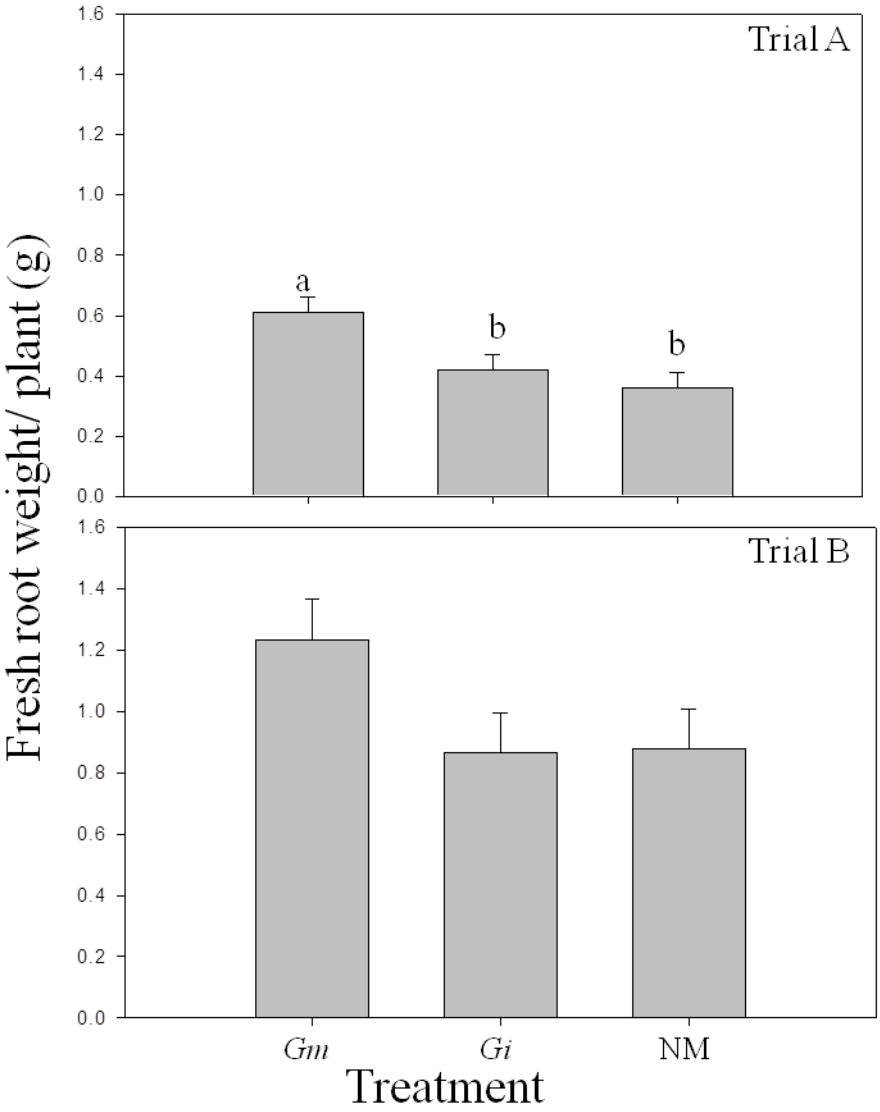

Fig. 3.6. Effect of mycorrhizae on fresh root weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (P= 0.0193, Trial A; P = 0.1140, Trial B).

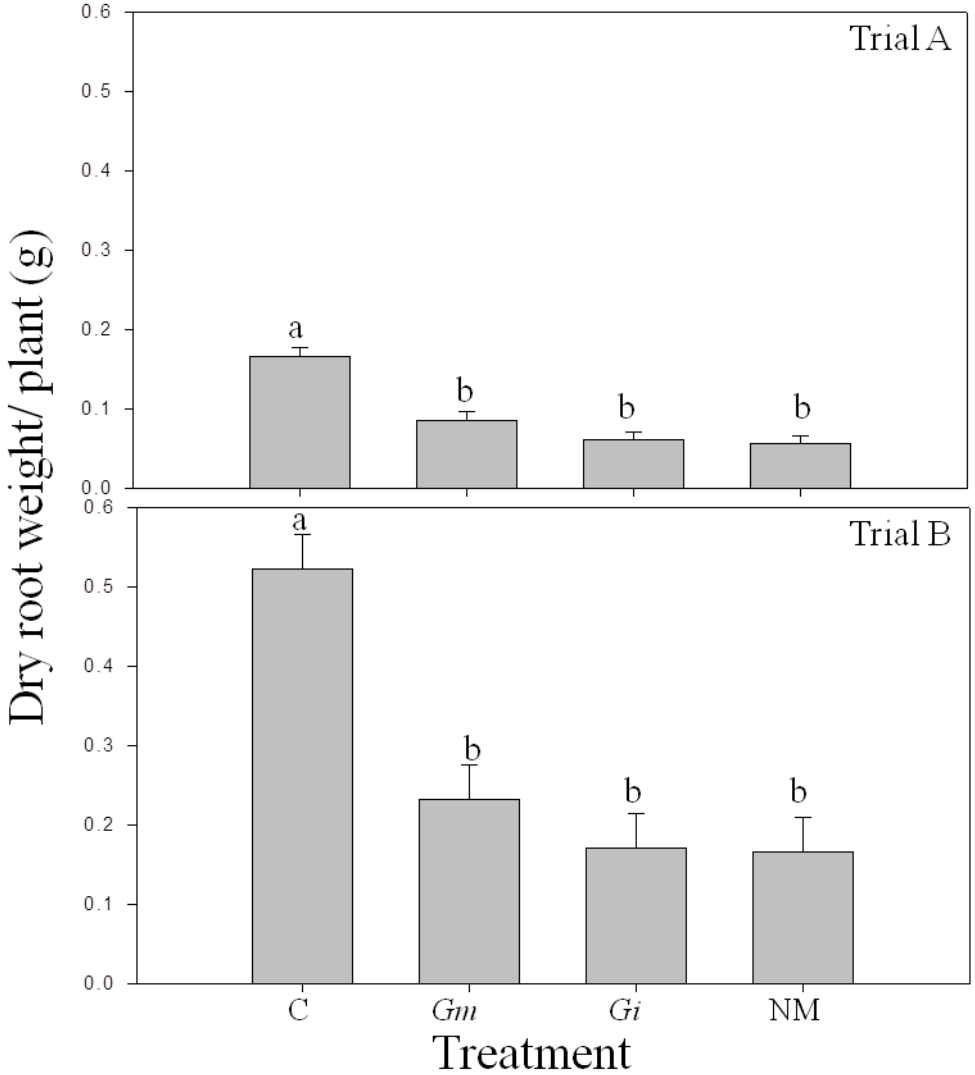

Fig. 3.7. Effect of mycorrhizae on dry root weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter are not different according to an F-protected LSD (*P*= 0.0001 for both trials).

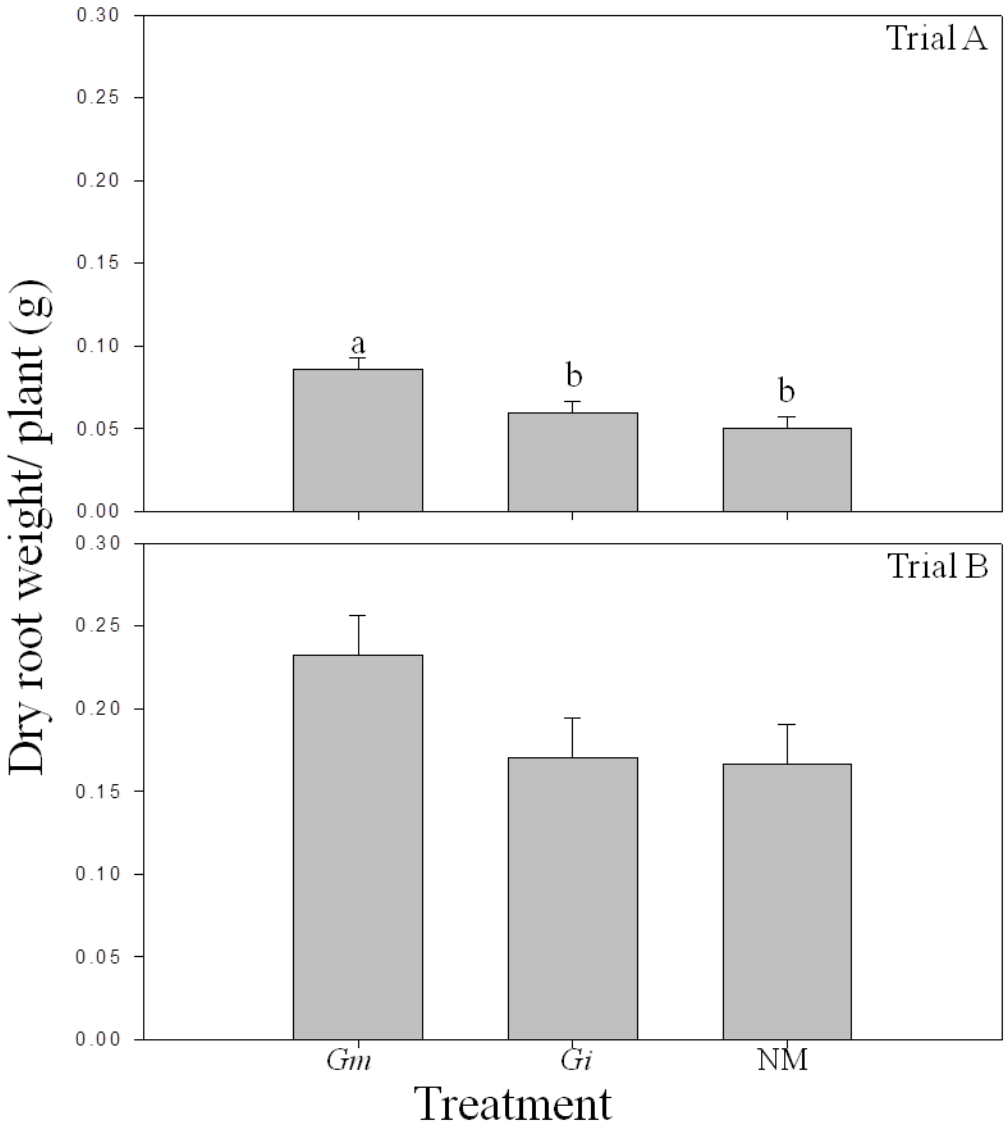

Fig. 3.8. Effect of mycorrhizae on dry root weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: non-mycorrhizal sorghum (NM); sorghum colonized with *Glomus intraradices* (*Gi*); and sorghum colonized with *Gigaspora margarita* (*Gm*). Wihin each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.0116, Trial A; *P*= 0.1266, Trial B).

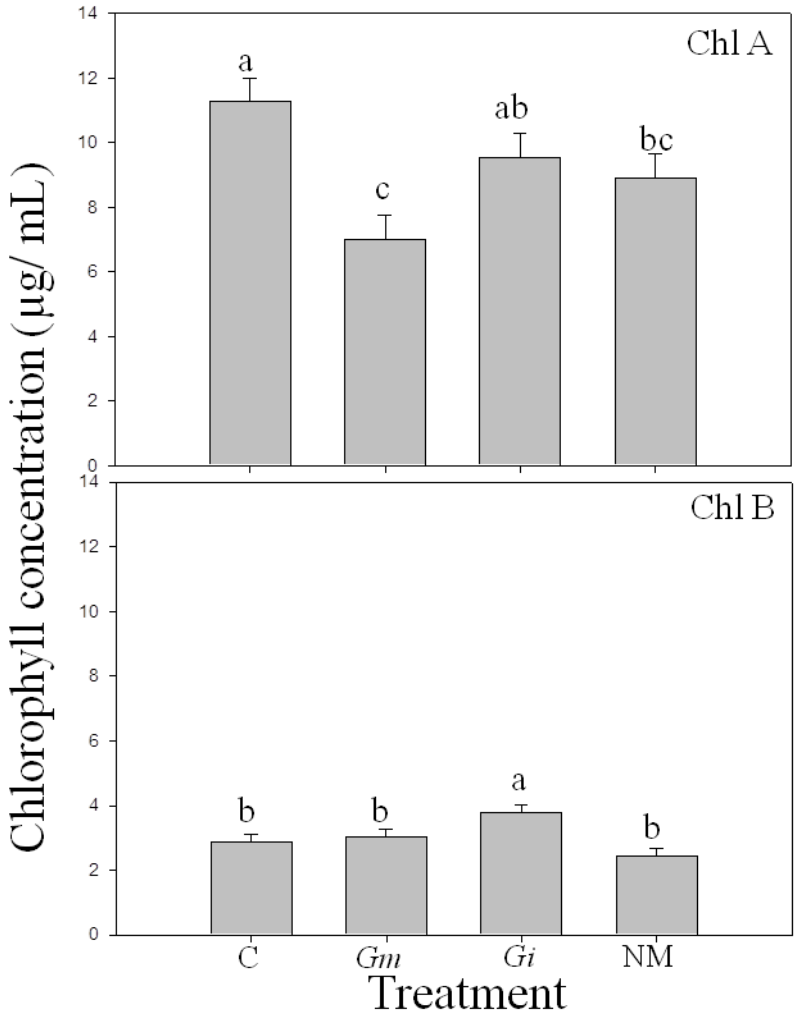

Fig. 3.9. Effect of mycorrhizae on chlorophyll A and B (µg/mL) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter are not different according to an F-protected LSD (*P*= 0.0052, Chl A; *P*= 0.0077, Chl B).

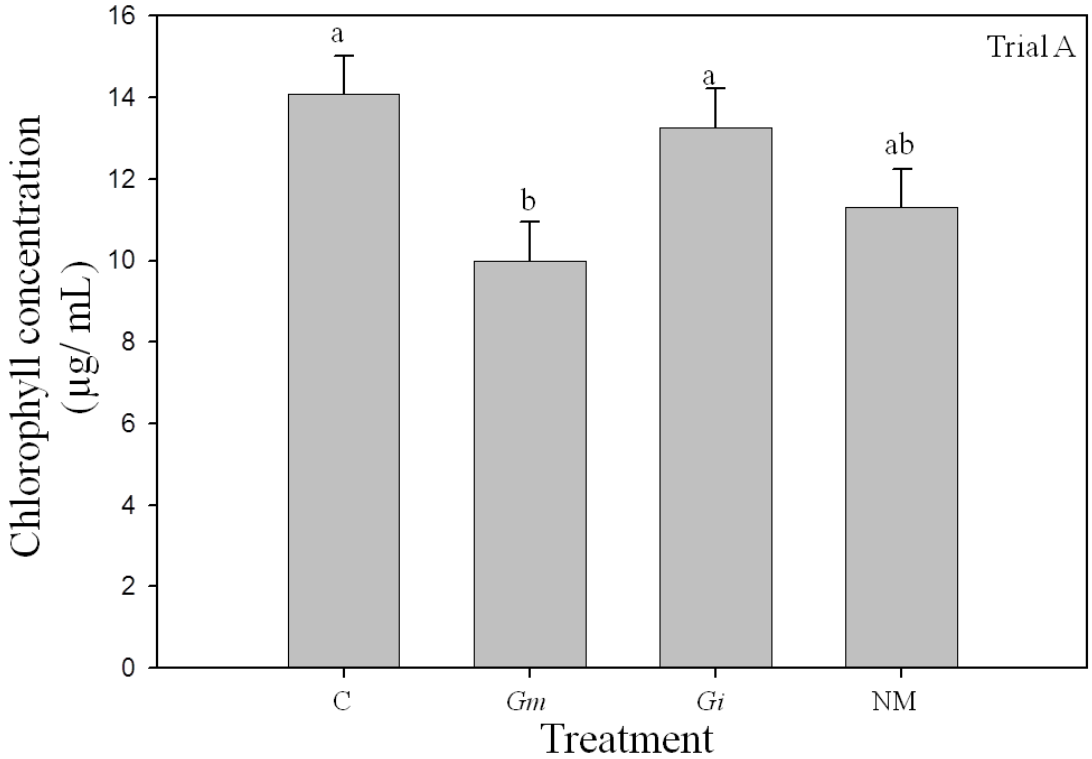

Fig. 3.10. Effect of mycorrhizae on concentration of total chlorophyll (A+B) (µg/mL) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.0253).

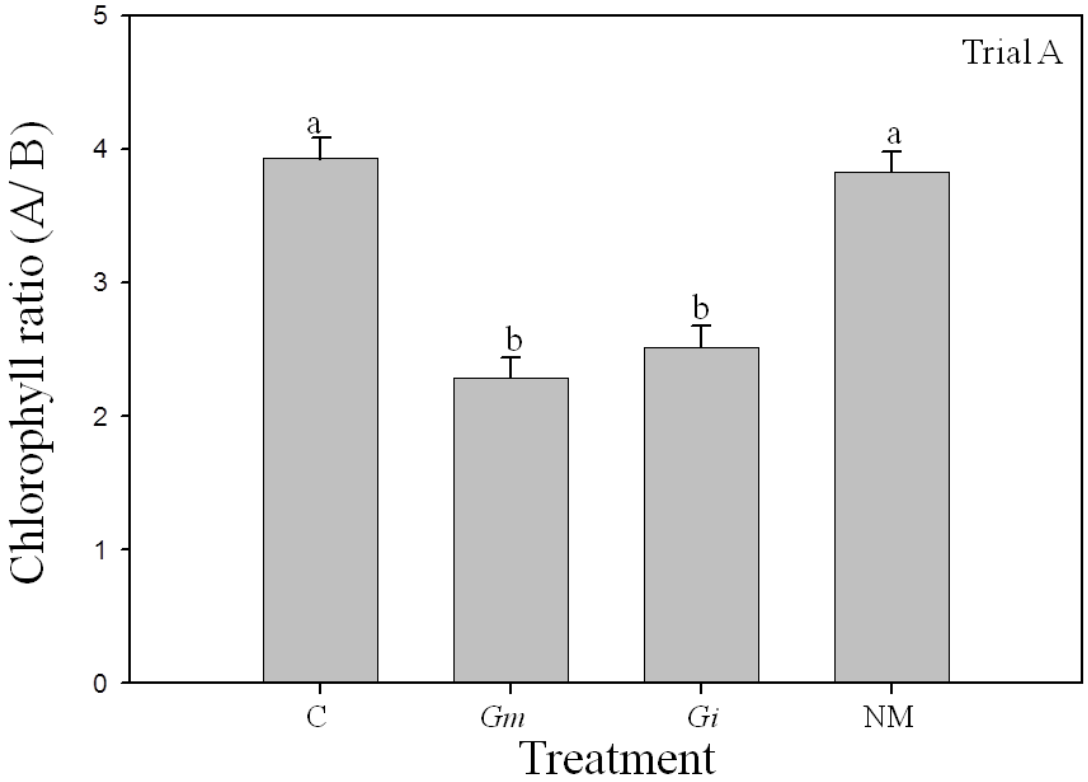

Fig. 3.11. Effect of mycorrhizae on chlorophyll A to Chlorophyll B (A/B) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Bars with the same letter are not different according to an F-protected LSD (P = 0.0001).

3.2. Effect of mycorrhizae on allelopathy (sorghum x Sudangrass hybrid)

To further investigate the effects of mycorrhizae on allelopathy, a sorghum x Sudangrass hybrid previously reported to produce large quantities of sorgoleone (Dayan et al. 2009) was used as the propagative host for the AM and NM cultures. In both trials, colonization level of wheat roots with *Gm* was low; colonization of wheat roots by *Gi* was slightly higher in both trials (Table 3.2).

Table 3.2. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings in allelopathy trials (sorghum x Sudangrass hybrid). Treatments are inocula obtained from sorghum x Sudangrass hybrid plants colonized with *Gigaspora margarita* (*Gm*) or sorghum x Sudangrass hybrid plants colonized with *Glomus intraradices* (*Gi*).

Treatment	Trial	AC (%)	VC (%)	HC (%)
Gm	A	4	.	17
Gi	A	17	1	23.6
Gm	B	2	.	19
Gi	B	5	8	12

Summary of statistical values (F-values, *P*-values and degrees of freedom) for all trials can be found in Appendix 1 (Table A.2).

Control plants had the greatest shoot height compared to all other treatments in both Trials A and B (Fig. 3.12). Height of wheat plants colonized by *Gm* or *Gi* was significantly higher than non-mycorrhizal plants in Trial A (Fig. 3.12 A) but not in Trial B (Fig. 3.12 B). Control plants were removed from the analysis to find out if there is a difference between mycorrhizal and non-mycorrhizal plants in shoot height (Fig. 3.13). Height of wheat plants colonized by *Gm* and *Gi* was greater than non-mycorrhizal plants in Trial A, but there was no difference among the treatments in Trial B (Fig. 3.13).

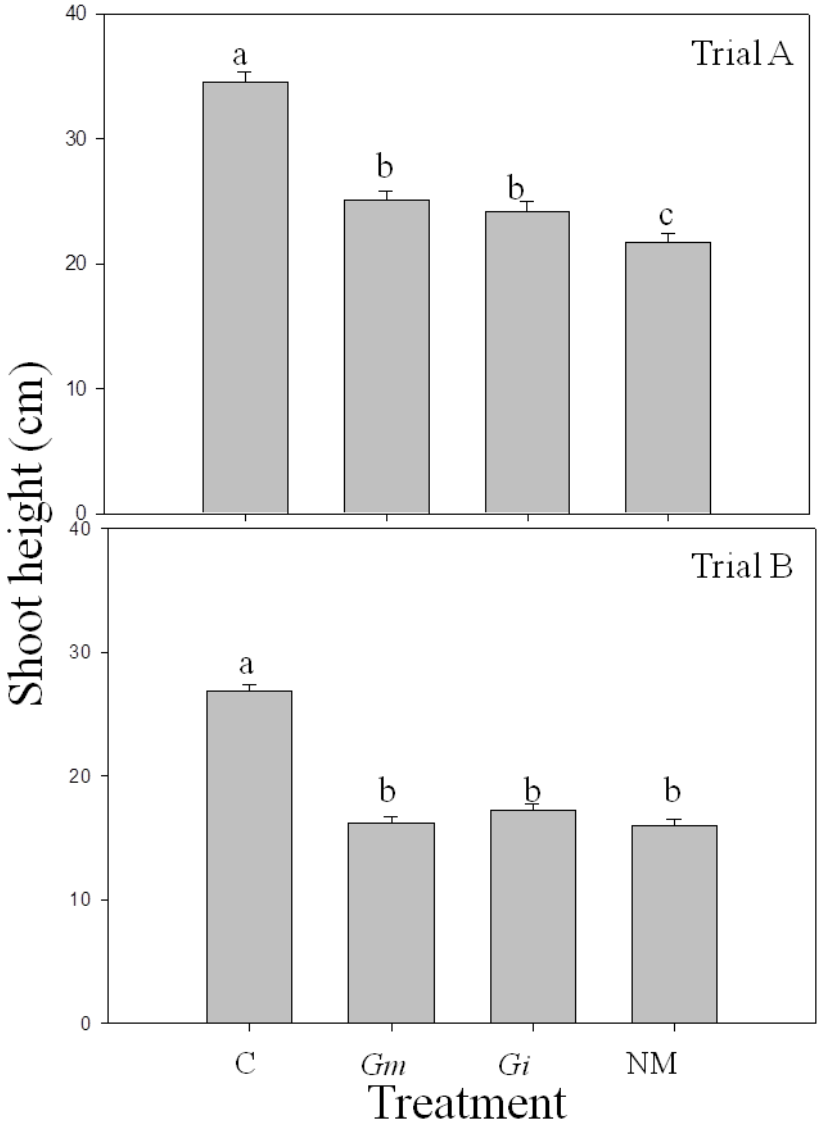

Fig. 3.12. Effect of mycorrhizae on shoot height (cm). Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: control (no-sorghum hybrid, no-mycorrhizae) (C); sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.0001 for both trials).

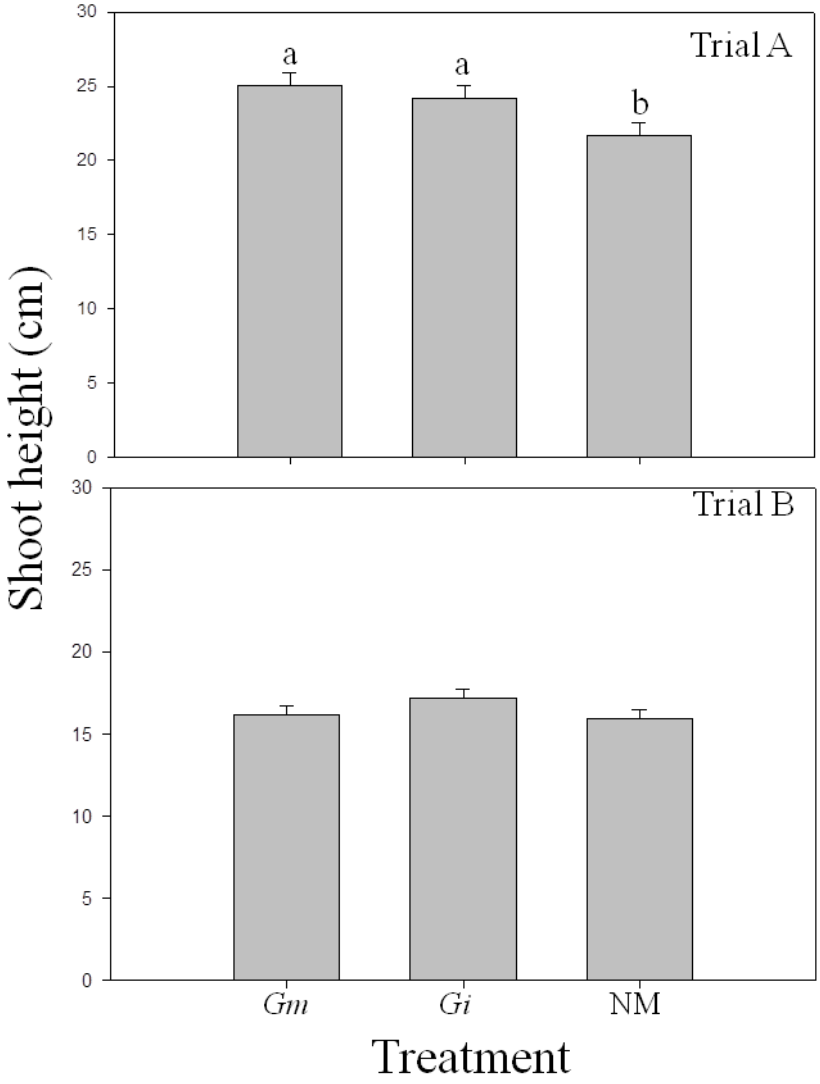

Fig. 3.13. Effect of mycorrhizae on shoot height (cm). Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (P = 0.0275, Trial A; P= 0.3432, Trial B).

Control wheat plants had the greatest fresh shoot weight, and plants in the nonmycorrhizal treatment had greater weights than plants colonized with *G. intraradices* (Fig. 3.14) in both trials. When control plants were removed from the analysis, non-mycorrhizal and *Gm* plants were not different from each other in both trials. There was no difference between the two mycorrhizal isolates in Trial A (Fig. 3.15); however, both NM and *Gm* plants had greater fresh shoot weight than *Gi* plants in Trial B (Fig. 3.15).

Control plants had greater stem diameter than plants that received the NM or mycorrhizal treatments (Fig. 3.16) in Trial A. Mycorrhizal wheat plants did not differ from their non-mycorrhizal counterparts (NM) (Fig. 3.16). Stem diameters were not measured in Trial B.

Fresh root weights were not different among treatments in either trial (Fig. 3.17). Dry root weights of plants in the NM treatment were greater than those in the control and the *Gi* treatments (Fig. 3.18). Dry root weights were not measured in Trial B. When control plants were removed from the analysis, non-mycorrhizal plants had larger dry root weights than *Gi* plants, but *Gm* plants were not different from either NM or *Gi* treatments ($P < 0.013$) (Fig. 3.19).

Plants colonized with *Gi* had lower concentrations of Chlorophyll A than all other treatments (Fig. 3.20A). Control and *Gm* treatments had greater concentrations of Chlorphyll B than NM and *Gi* (Fig. 3.20B). Total chlorophyll (Chl A+B) was greater in the no-sorghum hybrid control and *Gm* treatments than in *Gi* treatments (Fig. 3.21). No non-mycorrhizal (NM) plants had lower ratios of Chlorophyll A content to Chlorophyll B content (Chl A/ B) than plants in all other treatments (Fig. 3.22). No difference was observed between the two mycorrhizal isolates.

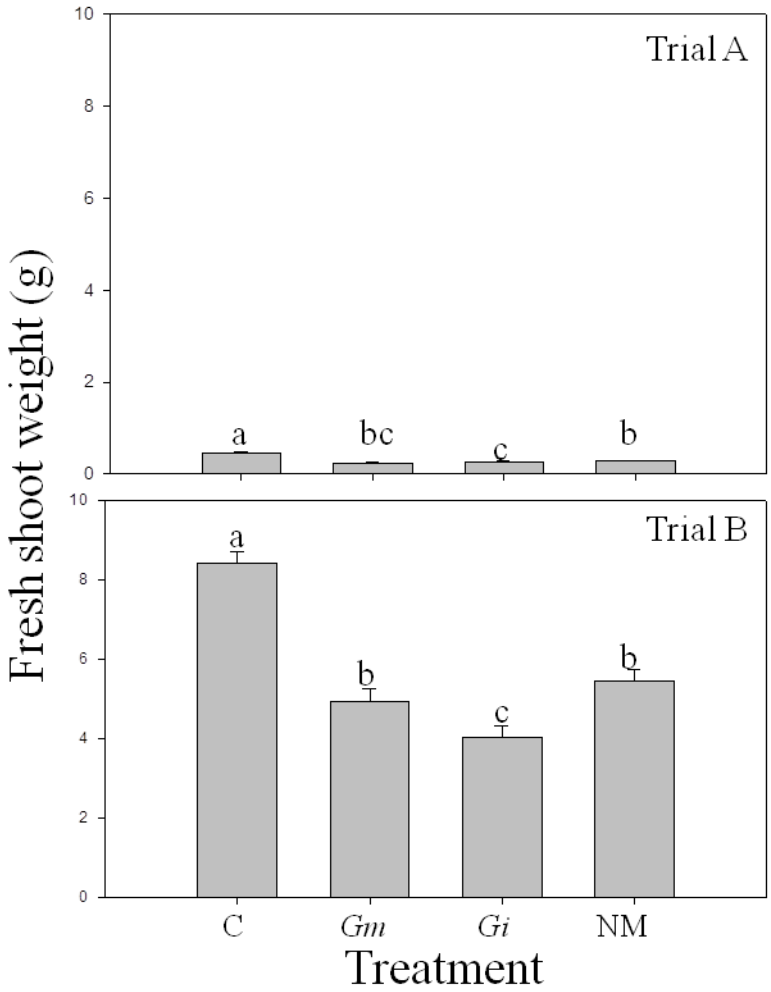

Fig. 3.14. Effect of mycorrhizae on fresh shoot weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: control (no-sorghum hybrid, no-mycorrhizae) (C); sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Within each trial, bars with the same letter are not different according to an F-protected LSD (*P*= 0.0001 for both trials).

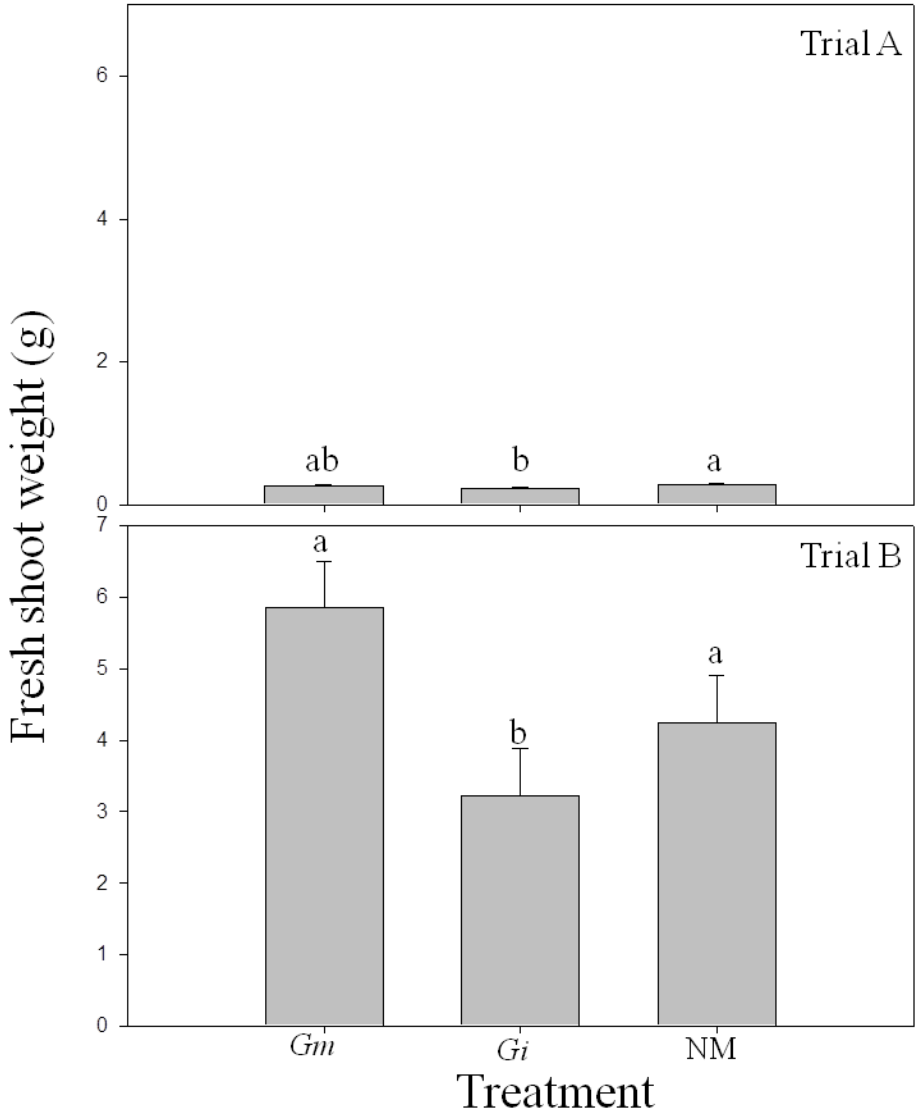

Fig. 3.15. Effect of mycorrhizae on fresh shoot weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Within each trial, bars with the same letter are not different according to an F-protected LSD (P = 0.0315, Trial A; P = 0.0001, Trial B).

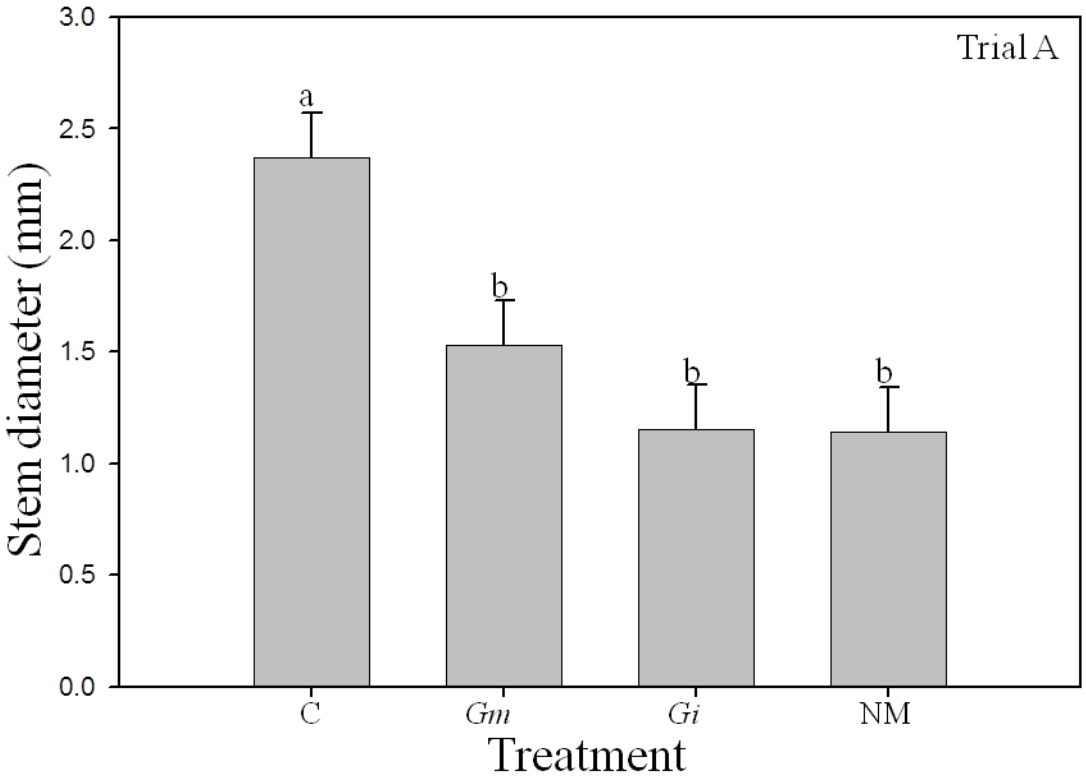

Fig. 3.16. Effect of mycorrhizae on stem diameter of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: control (no-sorghum hybrid, no-mycorrhizae) (C); sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Bars with the same letter are not different according to an F-protected LSD (P = 0.0009).

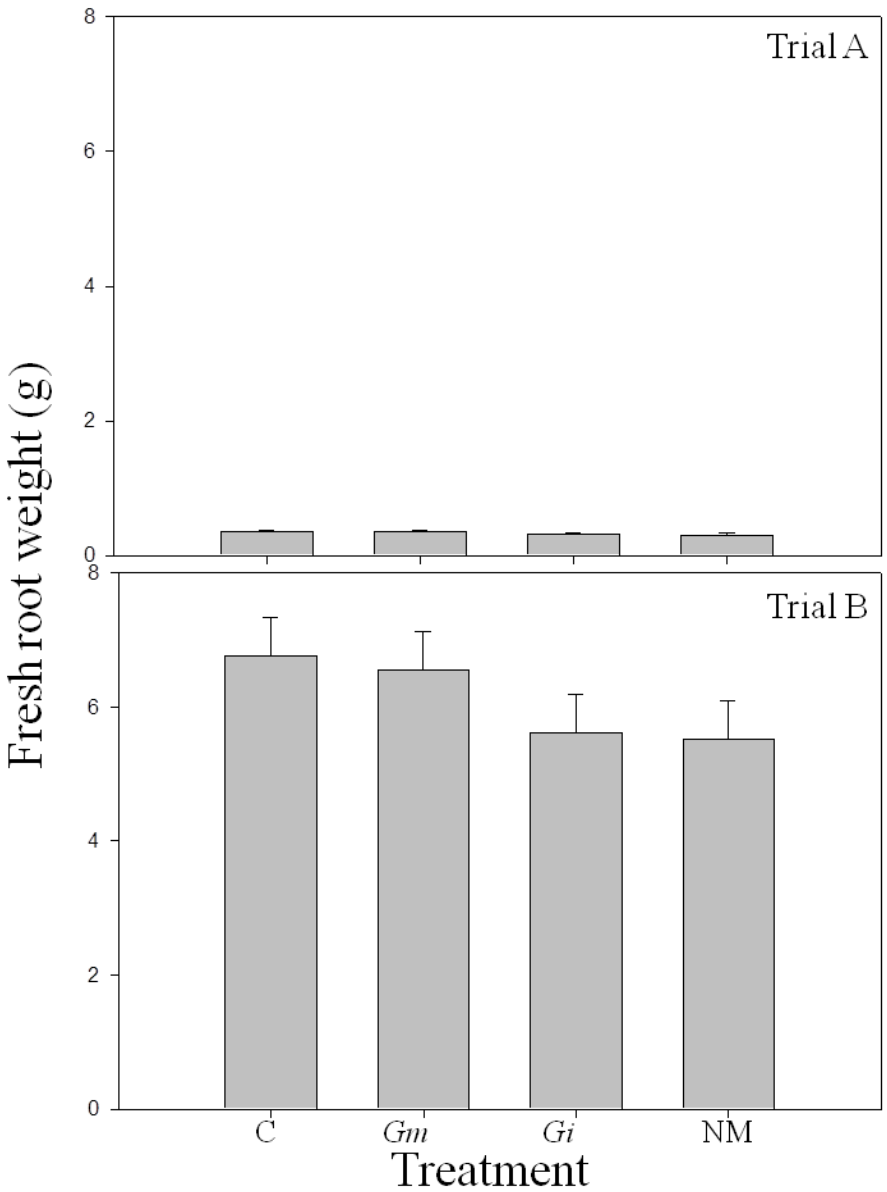

Fig. 3.17. Effect of mycorrhizae on fresh root weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: control (no-sorghum hybrid, no-mycorrhizae) (C); sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Within each trial, bars without letters are not different according to an F-protected LSD (P= 0.4728, Trial A; P = 0.3242, Trial B).

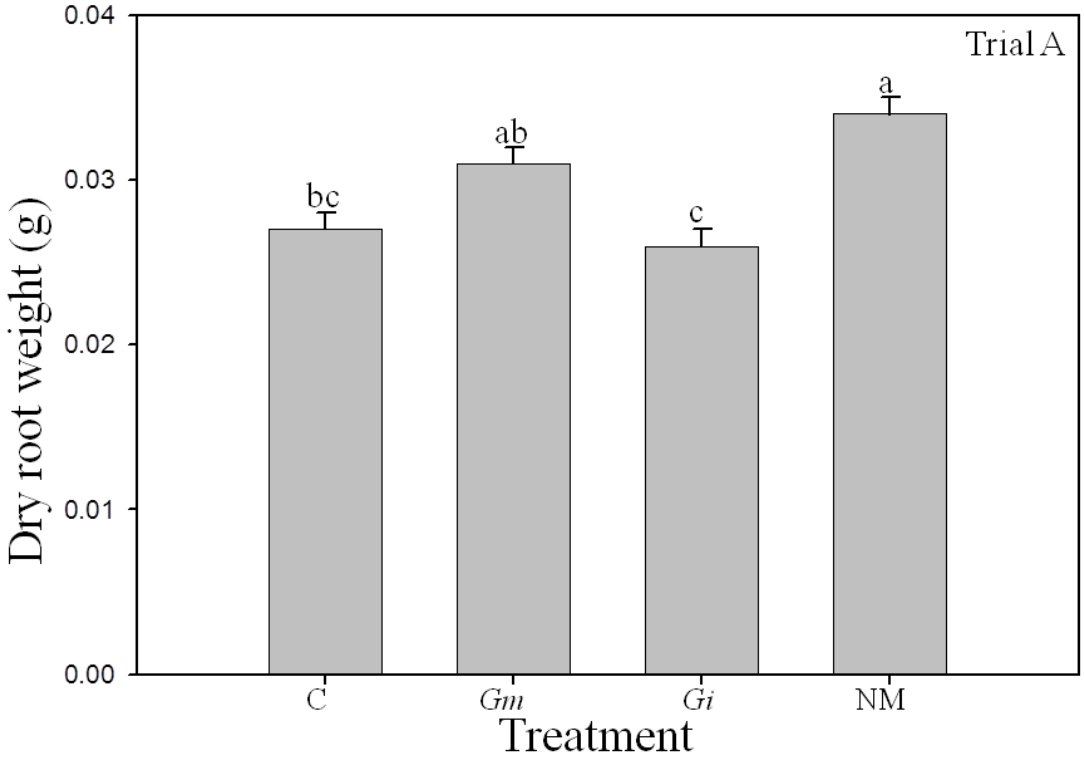

Fig. 3.18. Effect of mycorrhizae on dry root weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: control (no-sorghum hybrid, no-mycorrhizae) (C); sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.0092).

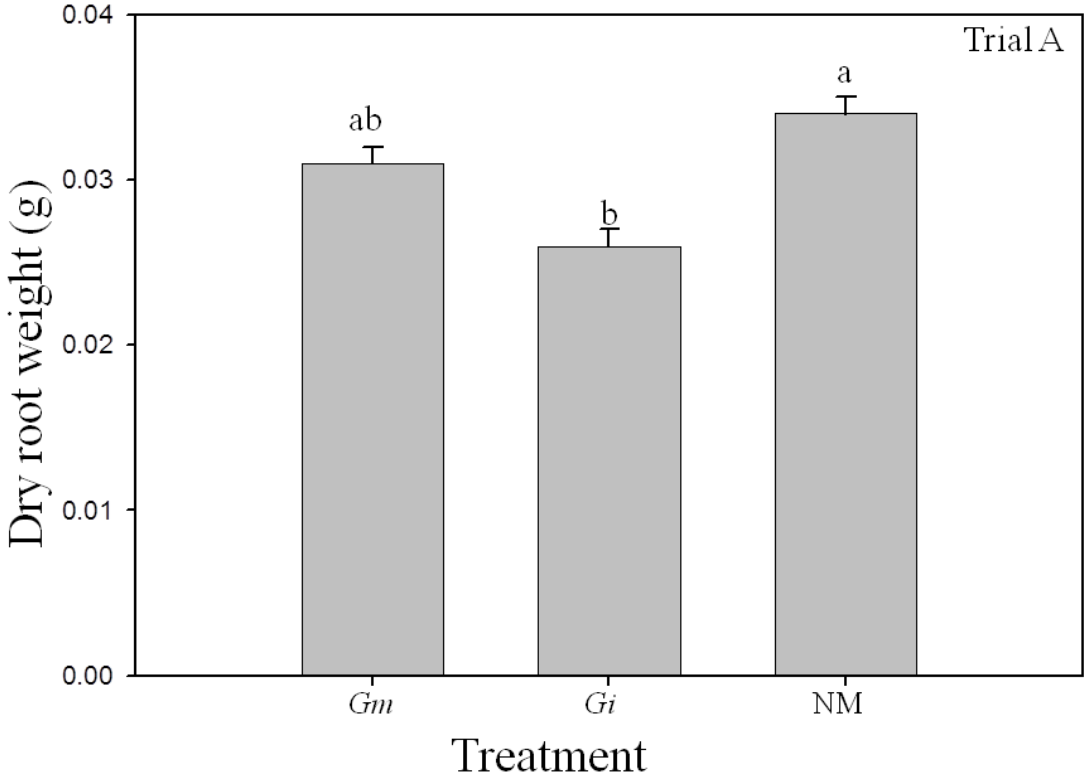

Fig. 3.19. Effect of mycorrhizae on dry root weight (g) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Bars with the same letter are not different according to an F-protected LSD (P = 0.0137).

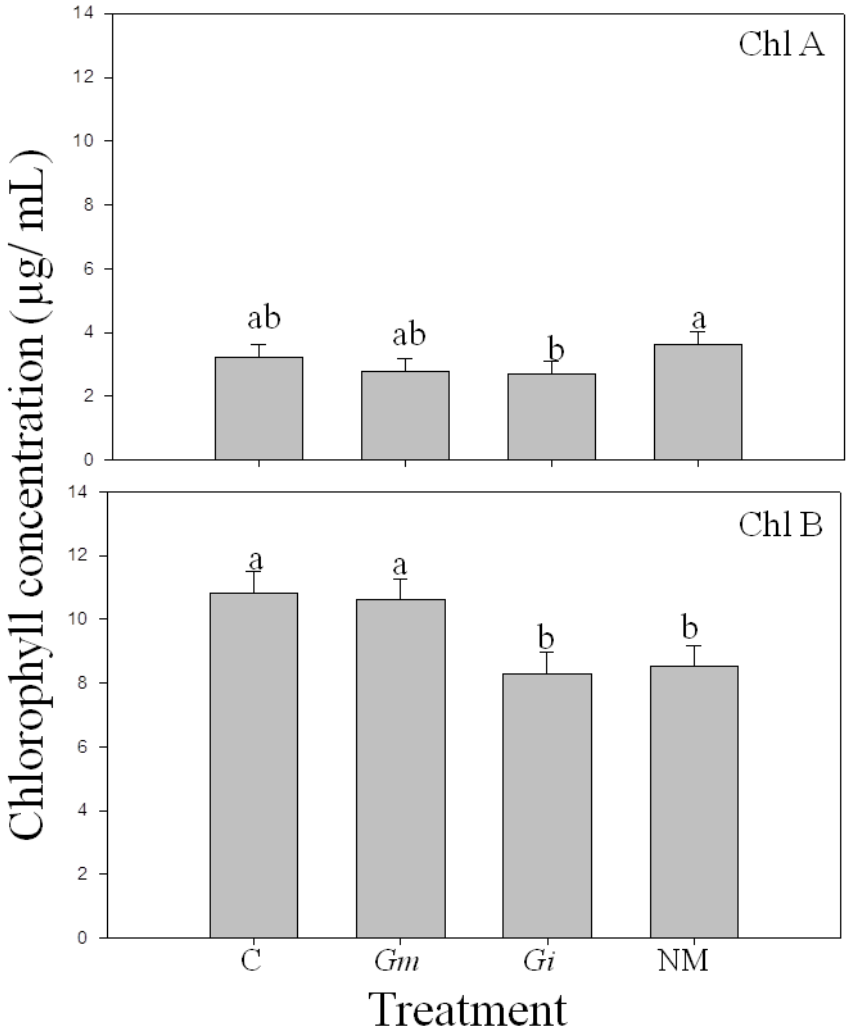

Fig. 3.20. Effect of mycorrhizae on chlorophyll A and B (µg/mL) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum-sudangrass hybrid with or without mycorrhizae. Treatments: control (no-sorghum hybrid, no-mycorrhizae) (C); sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Within each trial, bars with the same letter are not different according to an F-protected LSD (*P*= 0.0001 for both trials).

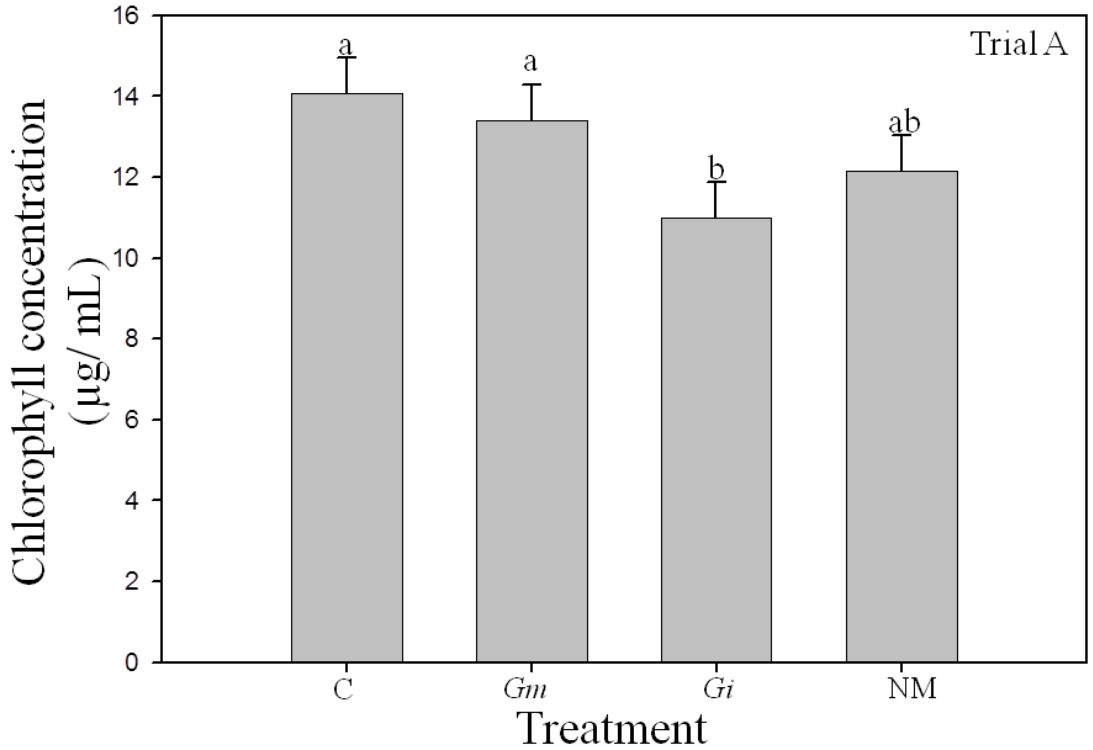

Fig. 3.21. Effect of mycorrhizae on concentration of total chlorophyll (A+B) (µg/mL) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: control (no-sorghum hybrid, no-mycorrhizae) (C); sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.0001)

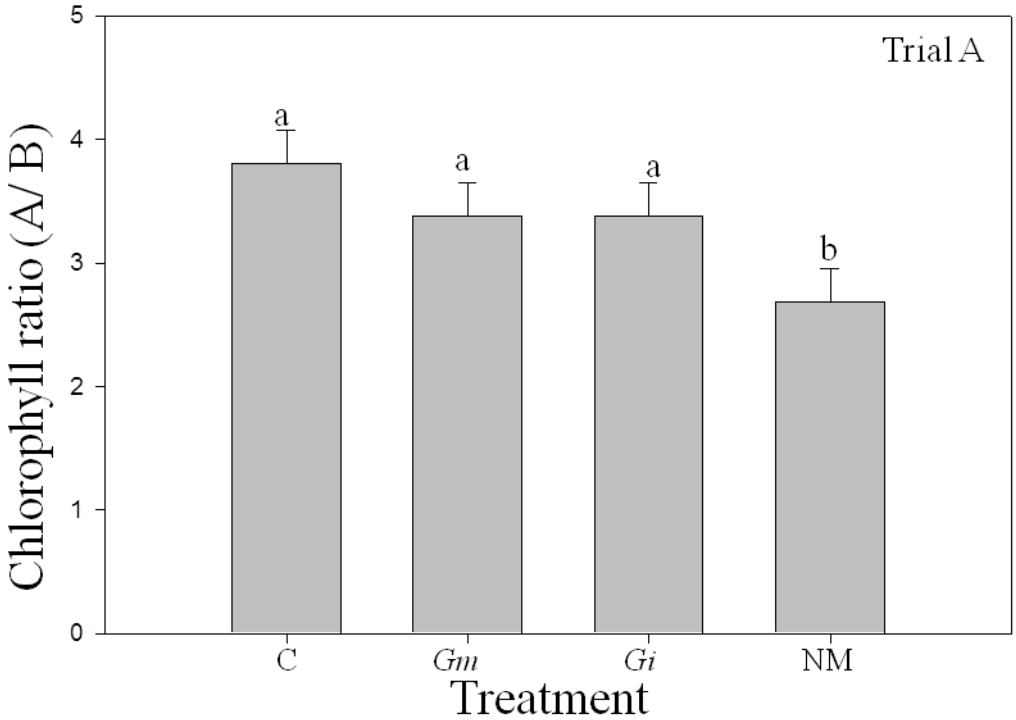

Fig. 3.22. Effect of mycorrhizae on the ratio of chlorophyll A to Chlorophyll B (A/B) of wheat. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum x Sudangrass hybrid with or without mycorrhizae. Treatments: control (no-sorghum hybrid, no-mycorrhizae) (C); sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM). Bars with same letter are not different according to an F-protected LSD (P = 0.0001).

3.3. Effect of mycorrhizae on aphid attraction

Natural Infestation. Aphids (*Rhopalosiphum padi*) were preferentially attracted to non-mycorrhizal plants in a natural infestation of the test plants in the greenhouse. Wheat plants colonized with *Gm* had no aphids, and plants colonized with *Gi* had few aphids is shown in Table 3.3.

In a second natural infestation, non-mycorrhzial (NM) wheat plants were heavily infested with aphids, but no insects were found on mycorrhizal or control plants when the mycorrhizae had been propagated on sorghum. Aphids were found on plants in

both the no-sorghum control and the NM treatment when the mycorrhizae were propagated on the sorghum x Sudangrass hybrid (Table 3.3).

Table 3.3. Number of aphids recorded in natural experiment of wheat seedlings. Mycorrhizae were propagated on either sorghum or a sorghum x Sudangrass hybrid. Treatments are: control (no-sorghum hybrid, no-mycorrhizae) (C); sorghum hybrid colonized with *Gigaspora margarita* (*Gm*); sorghum hybrid colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum hybrid (NM).

Natural infestation	Propagation host	Treatment	Aphid number
1	*S. bicolor*	C	.
1	*S. bicolor*	*Gm*	.
1	*S. bicolor*	*Gi*	15
1	*S. bicolor*	NM	300
2	*S. bicolor*	C	.
2	*S. bicolor*	*Gm*	.
2	*S. bicolor*	*Gi*	.
2	*S. bicolor*	NM	32.7
3	*S. bicolor*	C	.
3	*S. bicolor*	*Gm*	2
3	*S.bicolor*	*Gi*	.
3	*S.bicolor*	NM	46
2	Hybrid	C	28
2	Hybrid	*Gm*	.
2	Hybrid	*Gi*	.
2	Hybrid	NM	135.625

***Choice tests*.** Colonization of wheat seedlings with AM fungi is shown in Table 3.4.

Table 3.4. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings used in choice tests. Treatments are inocula obtained from either sorghum plants colonized with *Gigaspora margarita* (*Gm*) or sorghum colonized with *Glomus intraradices* (*Gi*).

Treatment	AC (%)	VC (%)	HC (%)
Gm	30	.	50
Gi	20	5	40

Summary of statistical values (F-values, *P*-values and degrees of freedom) for all trials can be found in Appendix 1 (Table A.3).

Seedling survival was higher in *Gm* than in *Gi* treatments, and there were no differences in survival between NM and *Gm* treatments for plants used in the choice trial (Fig. 3.23). Because there was a significant effect of treatment on seedling survival, all aphid counts were analyzed on a per plant basis.

Aphid numbers were not different between container types (jars vs pots) so data were combined (P= 0.245). There were no differences among the treatments in the choice experiment (Fig. 3.24) and no container x treatment interactions (P= 0.235).

Mycorrhizal plants colonized by *Gm* emitted larger amounts of butyronitrite, 2-ethylhexyl ester, and benzoic acid than their non-colonized counterparts (NM) (Fig. 3.25).

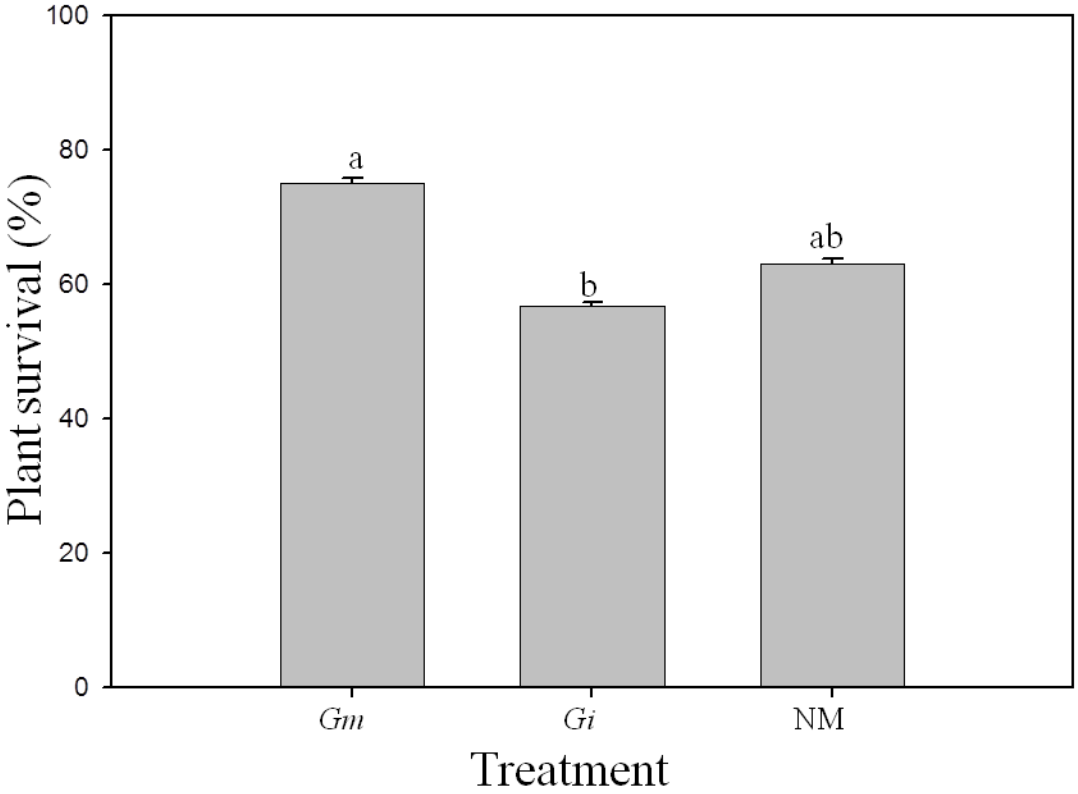

Fig. 3.23. Effect of mycorrhizae on survival of wheat seedlings used in choice tests. Twenty wheat seeds were planted in substrate containing sorghum with or without mycorrhizae for use in aphid choice tests. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.1070).

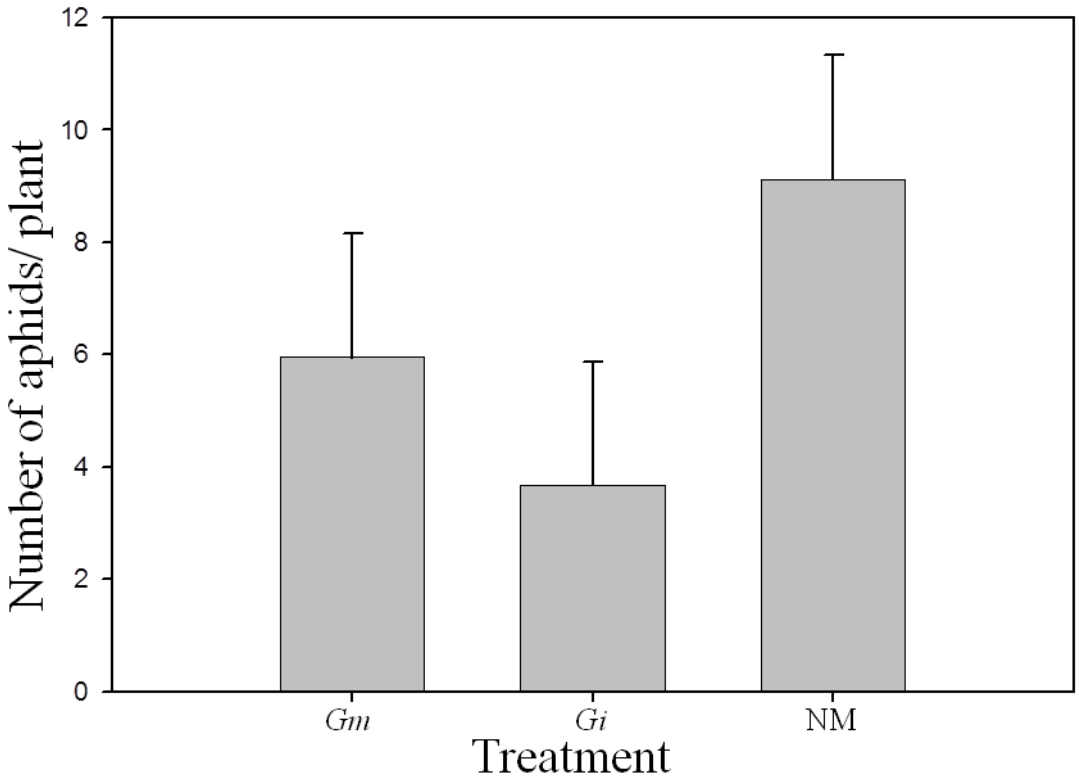

Fig. 3.24. Effect of mycorrhizae on aphid numbers on wheat seedlings (choice experiment). Aphids (*R. padi*) were collected and counted from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Source of aphids (a pot containing infested wheat) was placed equidistant from the treatments in an insect cage. After 5 days, aphids were counted. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Bars without letters are not different according to an F-protected LSD (*P*= 0.245).

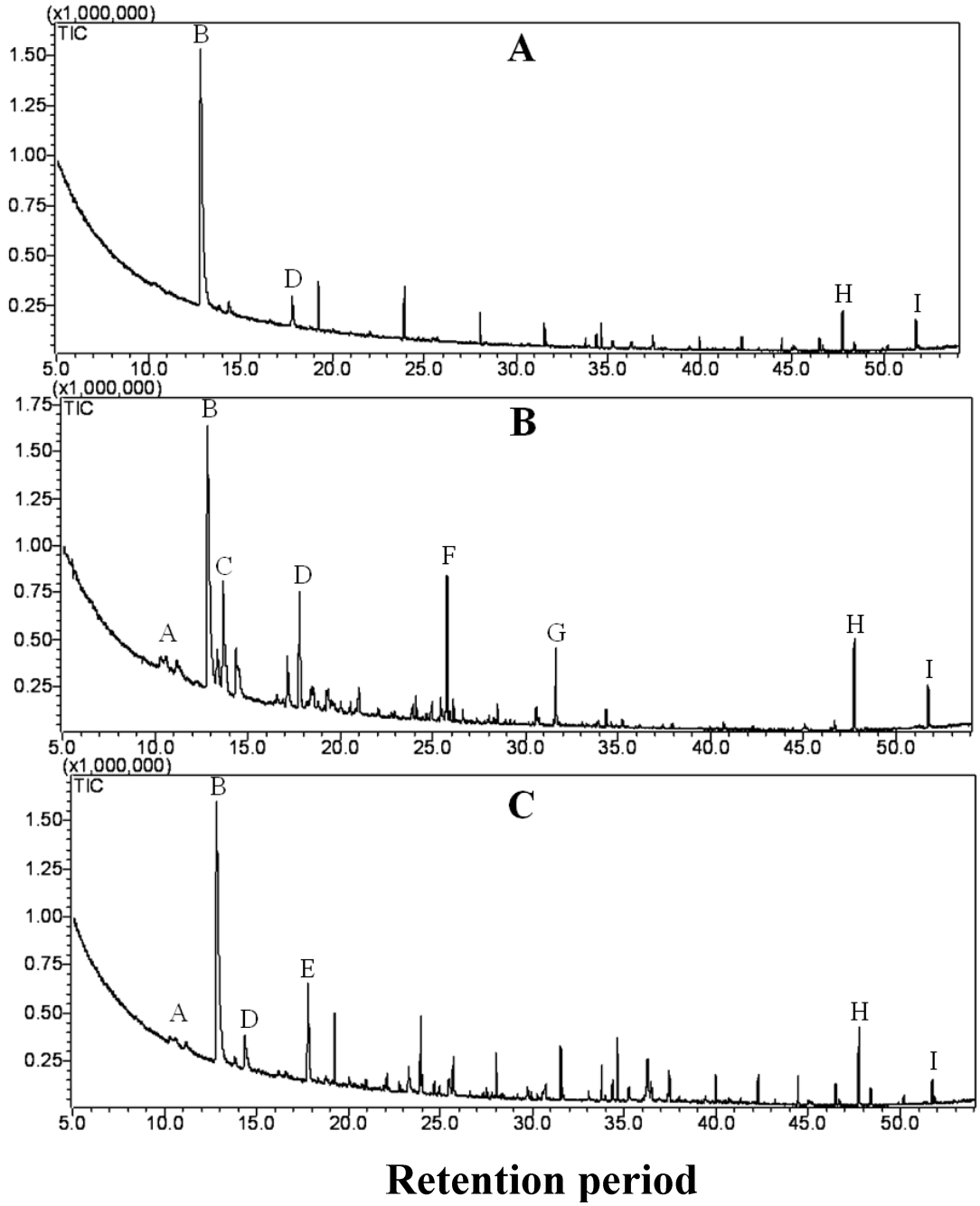

Fig. 3.25. Effect of mycorrhizae on volatiles of wheat (choice test). Volatiles from wheat seedling were collected and analyzed by Gas chromatography–mass spectrometry (GC-MS). The volatiles identified as 1- octanol (A), formic acid octyl ester (B), 3-hydroxy-3-phenyl butyronitrile (C), methylene chloride (D), chloromethyl octyl ether (E), 1,1'-oxybis octane (F), 2-ethylhexy ester benzoic acid (G), Di-n-octyl phthalate (H), 3,7,11-trimethyl 6, 10-dodecandien-3-01 (I). Seedlings were colonized with *Glomus intraradices* (A), colonized with *Gigaspora margarita* (B), or non-mycorrhizal sorghum (C).

***No-choice tests*.** Colonization of wheat seedlings with AM fungi is shown in Table 3.5.

Table 3.5. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings. Treatments are inocula obtained from either sorghum plants colonized with *Gigaspora margarita* (*Gm*) or sorghum colonized with *Glomus intraradices* (*Gi*).

Treatment	Trial	AC (%)	VC (%)	HC (%)
Gm	A	30	.	50
Gi	A	10	5	40
Gm	B	20	.	35
Gi	B	7	.	45

Summary of statistical values for all trials can be found in Appendix 1(Table A.4). There were no differences in seedling survival (Fig. 3.26 A), but there were differences among treatments for plant height and fresh shoot weight. Plant height was greater in non-mycorrhizal treatments than in *Gi* treatments; plants in the *Gm* treatment were not different from those in other treatments (Fig. 3.26 B). Fresh shoot weights of non-mycorrhizal and *Gm* plants were significantly greater than *Gi* plants (Fig. 3.26 C).

In no-choice experiments, aphid numbers/plant were significantly lower on *Gi* plants than on NM plants in both trials (Fig. 3.27); however, numbers of aphids on *Gm* plants were not different from those on either NM or *Gi*. Numbers in Trial A were approximately 7-times higher than in Trial B.

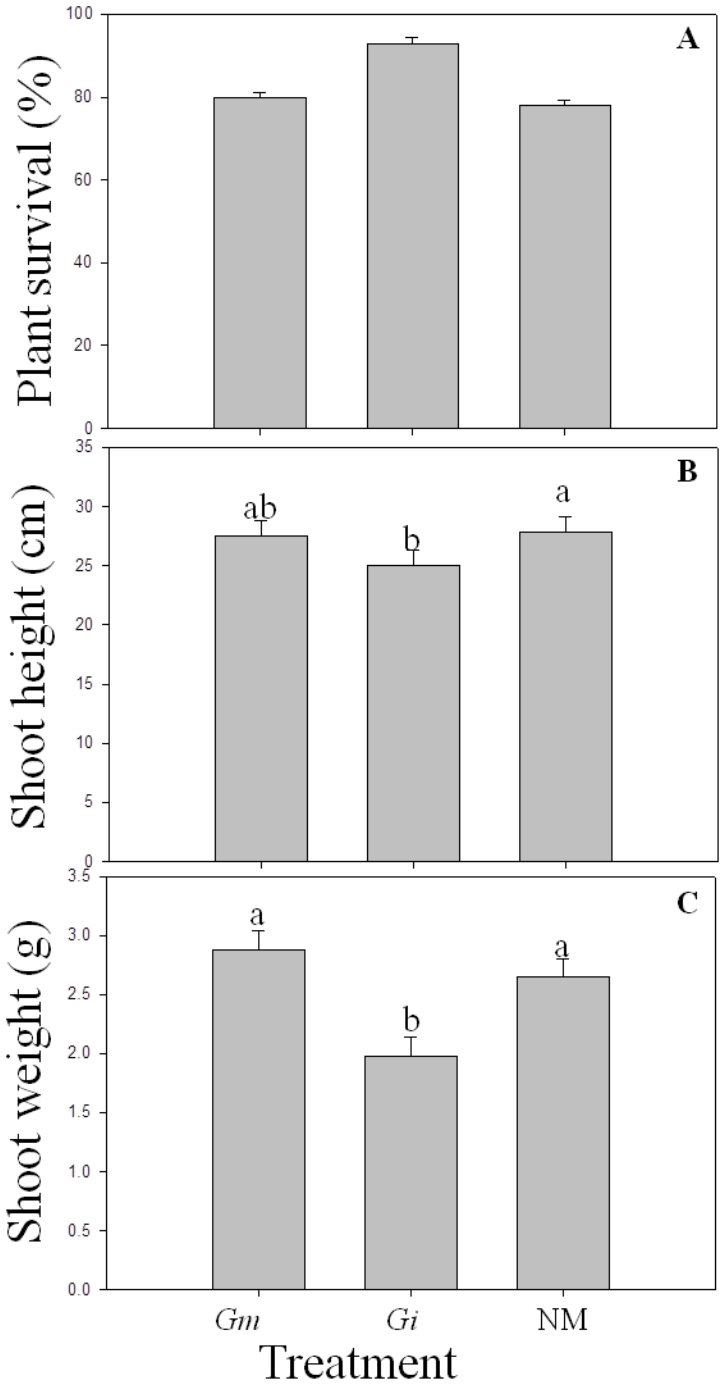

Fig. 3.26. Effect of mycorrhizae on plant survival, plant height (cm), and wheat weight (g) in no-choice test. Wheat seedlings were planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.2689, A; *P* = 0.0001, B; *P*= 0.0347, C)

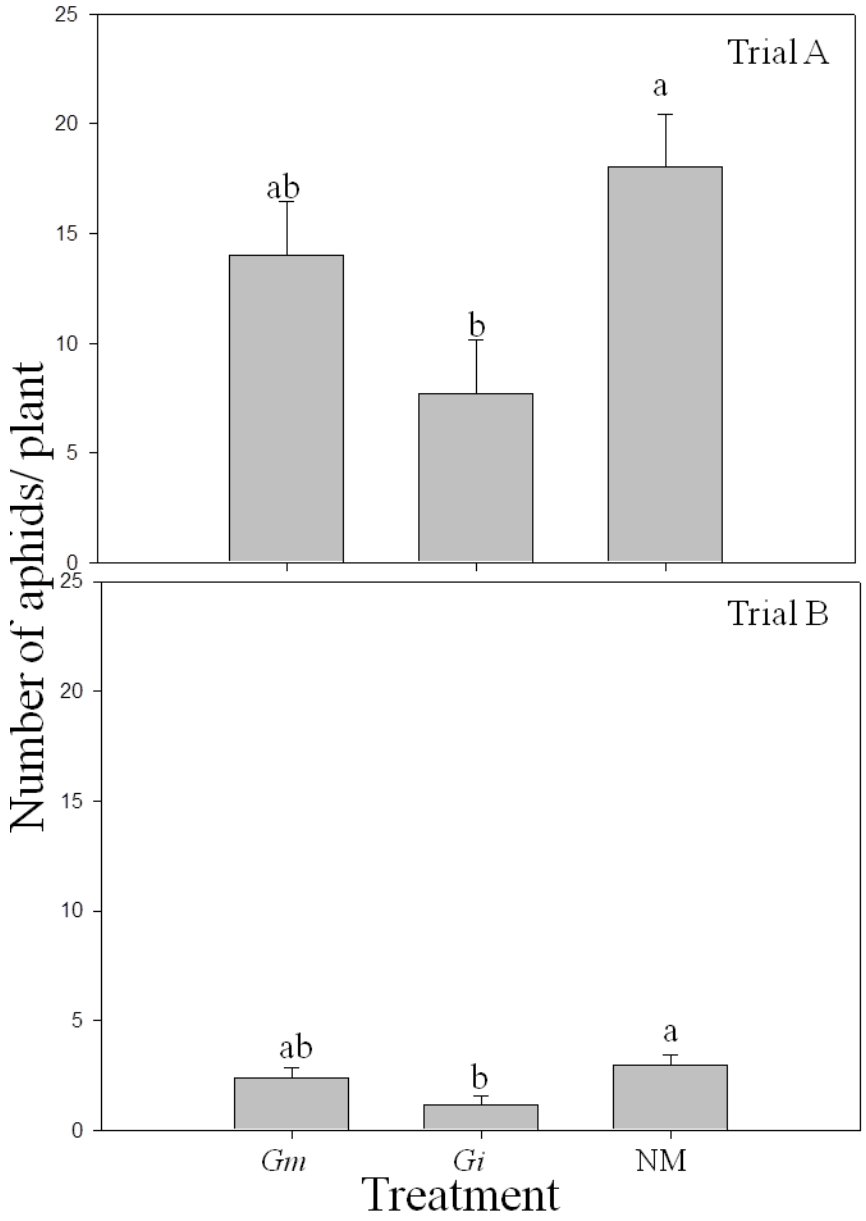

Fig. 3.27. Effect of mycorrhizae on aphid number of wheat in no-choice experiment. Twenty (*R. padi*) aphids were placed into each plant in all the treatments. Aphids were collected and counted from wheat seedlings planted and harvested after 4 weeks in substrate containing sorghum with or without mycorrhizae. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter are not different according to an F-protected LSD (*P*= 0.0912, Trial A; *P*= 0.0955, Trial B).

3.4. Fall armyworm (*Spodoptera frugiperda*) leaf assays.

Mycorrhizal colonization levels of wheat seedlings used in choice and no-choice experiments are shown in Table 3.6.

Table 3.6. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 4-week-old wheat seedlings used in fall armyworm feeding assays. Treatments are inocula obtained from either sorghum plants colonized with *Gigaspora margarita* (*Gm*) or sorghum colonized with *Glomus intraradices* (*Gi*).

Treatment	Trial	AC (%)	VC (%)	HC (%)
Gm	A	10	.	30
Gi	A	5	.	15
Gm	B	5	.	40
Gi	B	3	.	39

***Choice experiment* (*All treatments*).** More leaf surface was damaged in leaves from plants colonized by *Gi* leaves than were damaged in the no-sorghum, no-mycorrhizae control in Trial A (P= 0.030), but there were no differences were s among the treatments in Trial B (Fig. 3.28). When control leaves were excluded from the analysis in order to determine if there was a difference between mycorrhizal and non-mycorrhizal (NM) plants, damaged rating was not different between mycorrhizal and non-mycorrhizal (NM) leaves in either trial (Fig. 3.29). When the consumed leaf area was estimated by image analysis No difference was detected among the treatments in either trial (Fig. 3.30). When control leaves were excluded from the analysis, leaf consumption was not different among treatments in either trial (Fig. 3.31). When the image analysis damage estimate (%) was converted to a published feeding scale 0 to 3 (0 = no feeding; 3 = > 70% of leaf consumed) (Hardy et al., 1985), no significance difference was detected among the treatments in both trials (Fig. 3.32). When control leaves were excluded from the analysis, leaves from plants colonized by *Gi* were rated lower than the NM treatment in Trial A (Fig. 3.33). In contrast, no difference was seen in Trial B (Fig. 3.33).

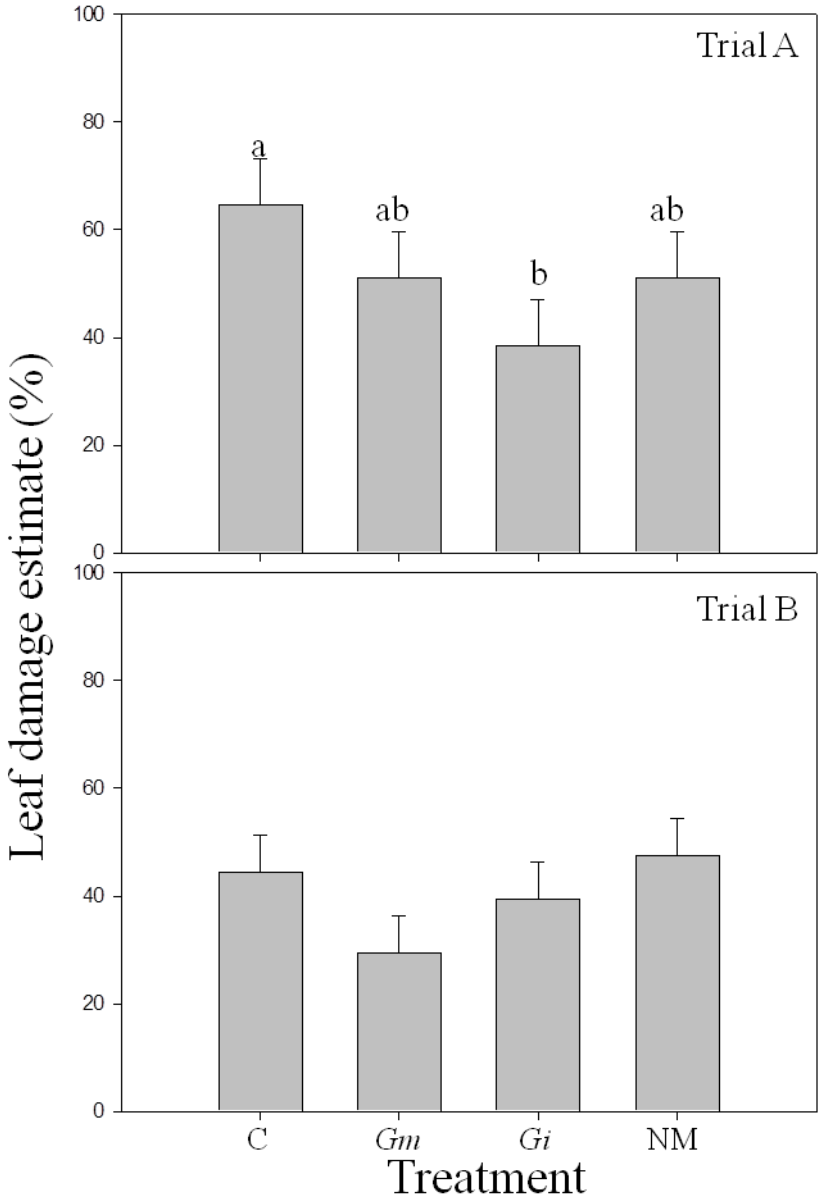

Fig. 3.28. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) – subjective estimate of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; the arena contained all treatments. Excised leaves obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Damage estimates are the mean of two raters' subjective scores. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.030, Trial A), (*P*= 0.298, Trial B).

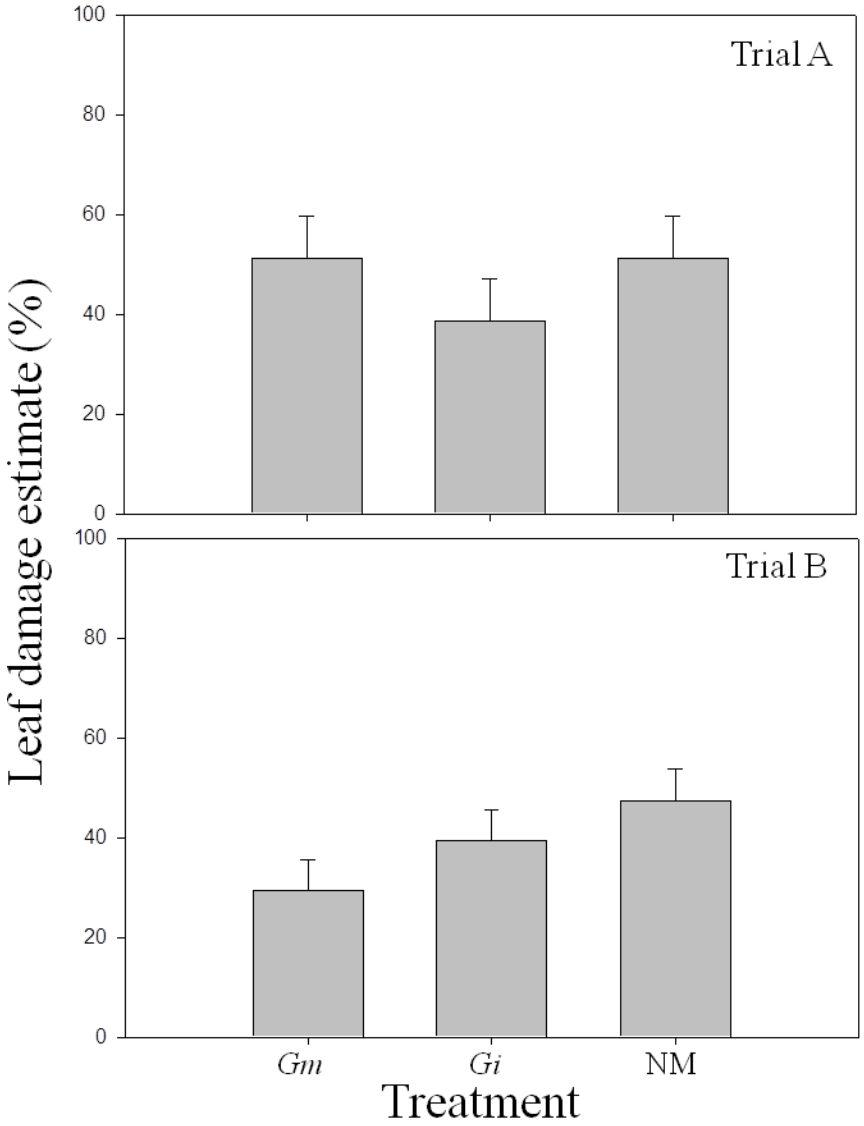

Fig. 3.29. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test)- subjective estimate of leaf damage (without control). Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; the arena contained all treatments. Excised leaves were obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Damage estimates are the mean of two raters' subjective scores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.297, Trial A), (*P*= 0.155, Trial B).

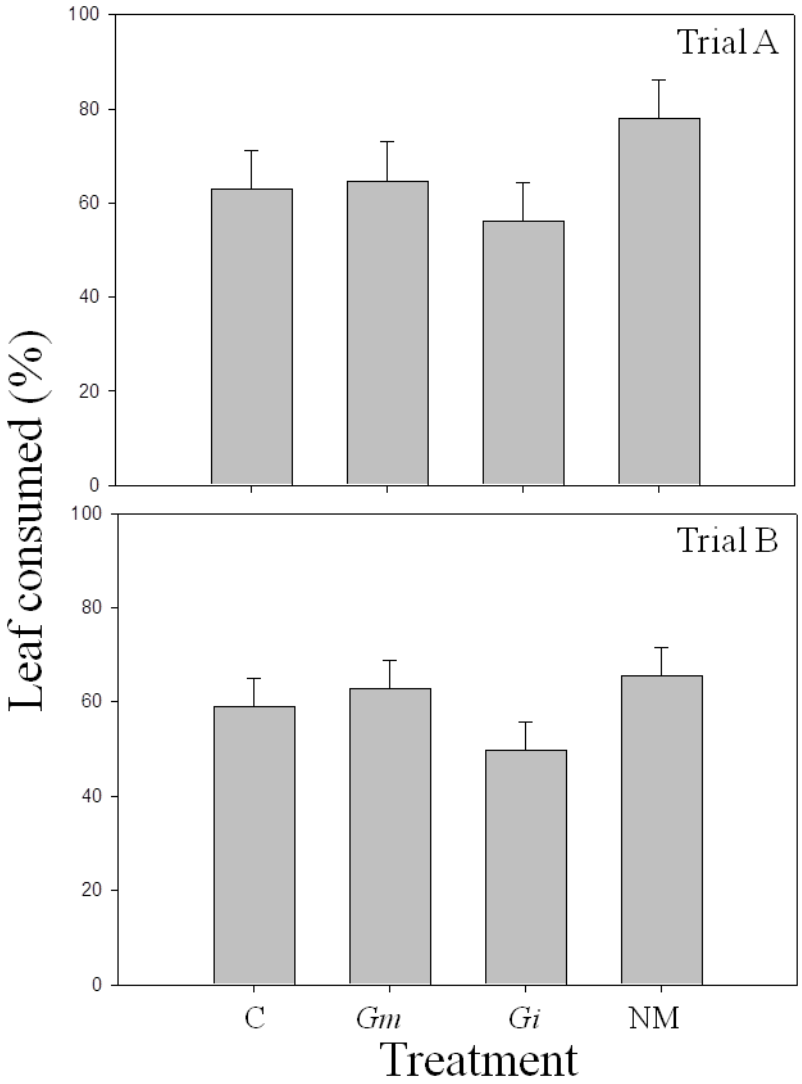

Fig. 3.30. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test)- image analysis of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; the arena contained all treatments. Excised leaves were obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Percentage consumption was determined using Assess 2.2 Image Analysis Software for Plant Disease Quantification. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.258, Trial A), (*P*= 0.267, Trial B).

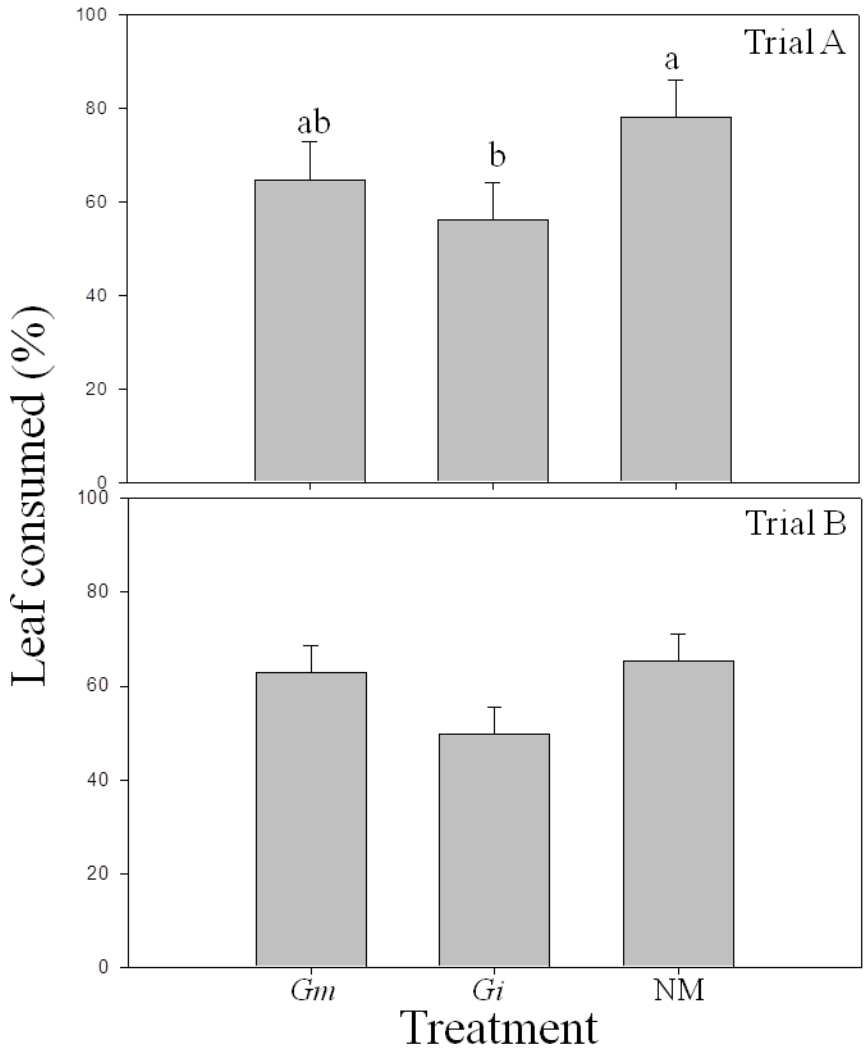

Fig. 3.31. Effect of mycorrhizae on fall armyworm (FAW) (Spodoptera frugiperda) feeding on wheat leaves (choice test) – image analysis of leaf damage (without control). Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; the arena contained all treatments. Excised leaves were obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Percentage consumption was determined using Assess 2.2 Image Analysis Software for Plant Disease Quantification. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.297, Trial A; *P*= 0.157, Trial B).

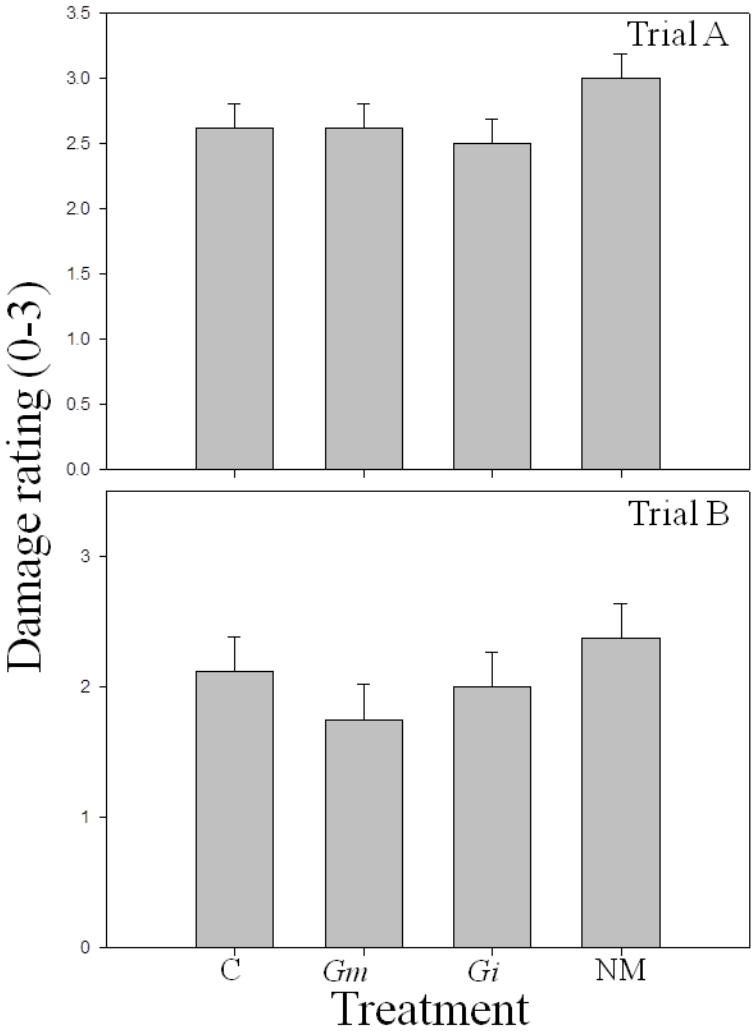

Fig. 3.32. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) –rating scale. Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; the arena contained all treatments. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Image analysis consumption estimate (%) was converted to the damage rating scale (0 – 3) developed by (Hardy et al., 1985). Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.252, Trial A; *P*= 0.442, Trial B).

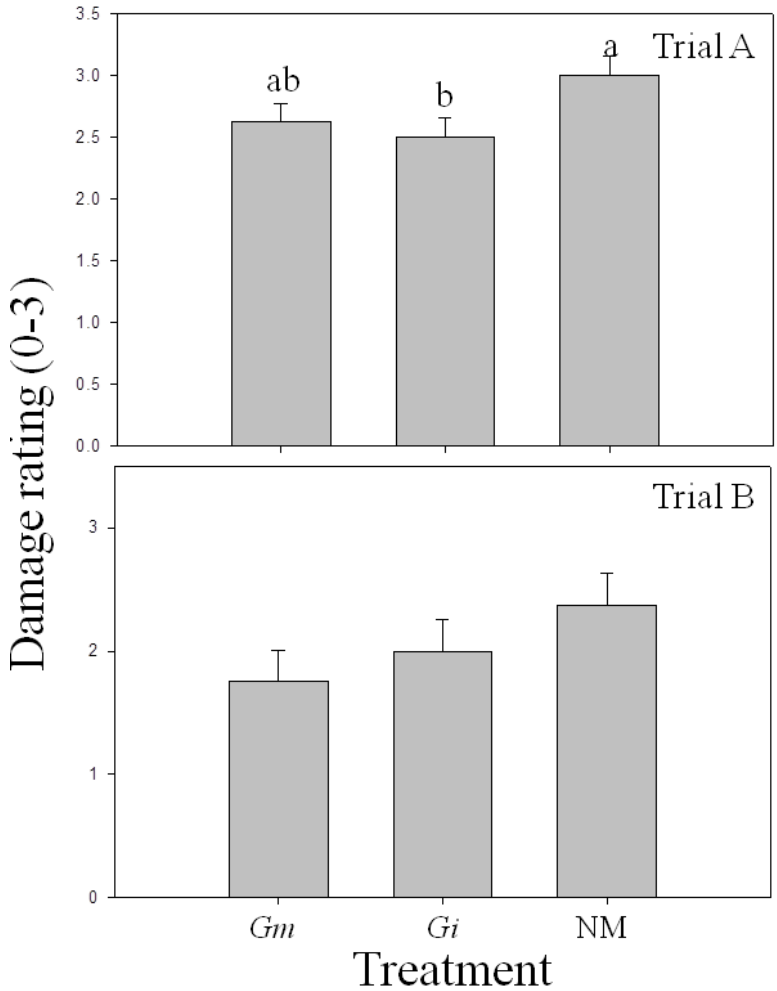

Fig. 3.33. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda)* feeding on wheat leaves (choice test) –rating scale (without control). Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; the arena contained all treatments. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Damage rating scale was 0 – 3 (Hardy et al., 1985). Image analysis consumption estimate (%) was converted to the damage rating scale (0 – 3) developed by (Hardy et al., 1985). Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.063, Trial A; *P*= 0.265, Trial B).

Choice experiment (pairwise comparison). No feeding parameters (larval survival, number of larvae feeding, feeding damage, feeding consumption, and damage rating) were different between the *Gm* and *Gi* treatments or between the NM treatments and either of the mycorrhizal treatments (Table 3.7).

Table 3.7. Effect of mycorrhizae on fall armyworm (*Spodoptera frugiperda*) feeding; values are P-values for a F-protected LSD. Twenty-five (*S. frugiperda*) larvae were placed equidistant from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Numbers of larvae were counted during five days. Treatments: no-sorghum, no-mycorrhizae control (C); and sorghum colonized with *Glomus intraradices* (*Gi*). NS = *P*-values > 0.100.

Trt 1	Trt 2	Larval survival (#)	Feeding larvae (#)	Non-feeding larvae (#)	Damage (%)	Consumed (%)	Rating
Control	*Gi*	NS	NS	NS	0.0529	0.0898	NS
Control	*Gm*	NS	NS	NS	0.0908	NS	NS
Control	NM	0.0669	NS	0.0650	NS	NS	NS
NM	*Gm*	NS	NS	NS	NS	NS	NS
NM	*Gi*	NS	NS	NS	NS	NS	NS
Gm	*Gi*	NS	NS	NS	NS	NS	NS

Percentages of leaf damage and leaf consumed were greater in control than in *Gi* (Figs. 3.34 and 3.35). Leaf damage was also greater in control than in *Gm* (Fig. 3.36). Larvae survival rate was greater in control than in NM (Fig. 3.37). Given the choice, the number of non-feeding larvae was greater in control than in non-mycorrhizal leaves (Fig. 3.38). When FAW larvae were provided leaves with any other combination of treatments, there were no differences; figures for other pairwise comparisons are in Appendix 3 (Figs. A.3 - A.18).

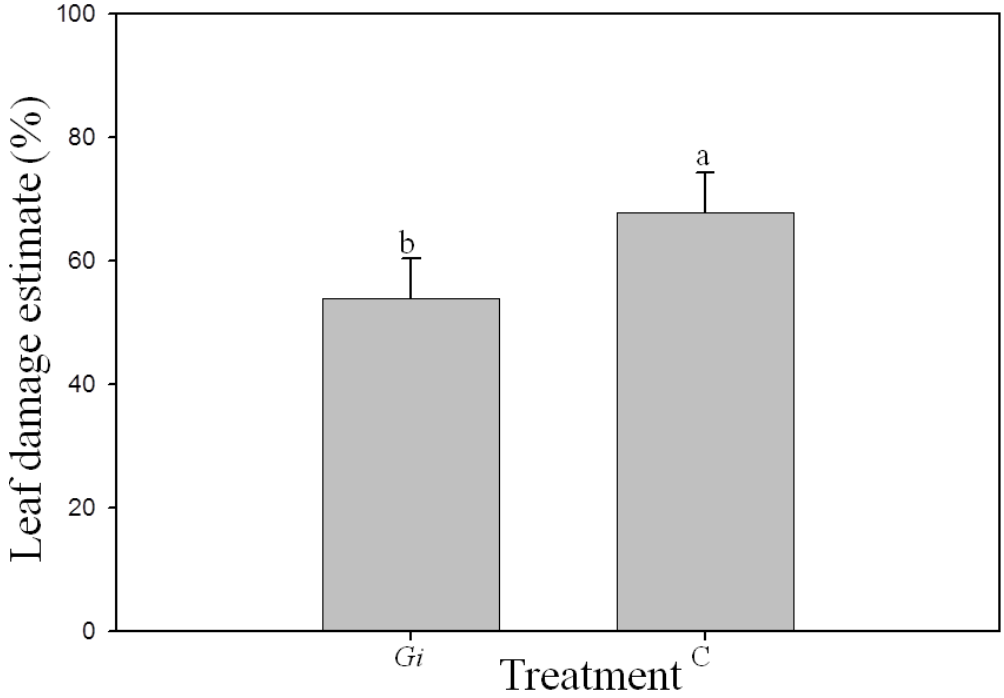

Fig. 3.34. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) – subjective estimate of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Damage estimates are the mean of two raters' subjective scores. Treatments: no-sorghum, no-mycorrhizae control (C); and sorghum colonized with *Glomus intraradices* (*Gi*). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.0529).

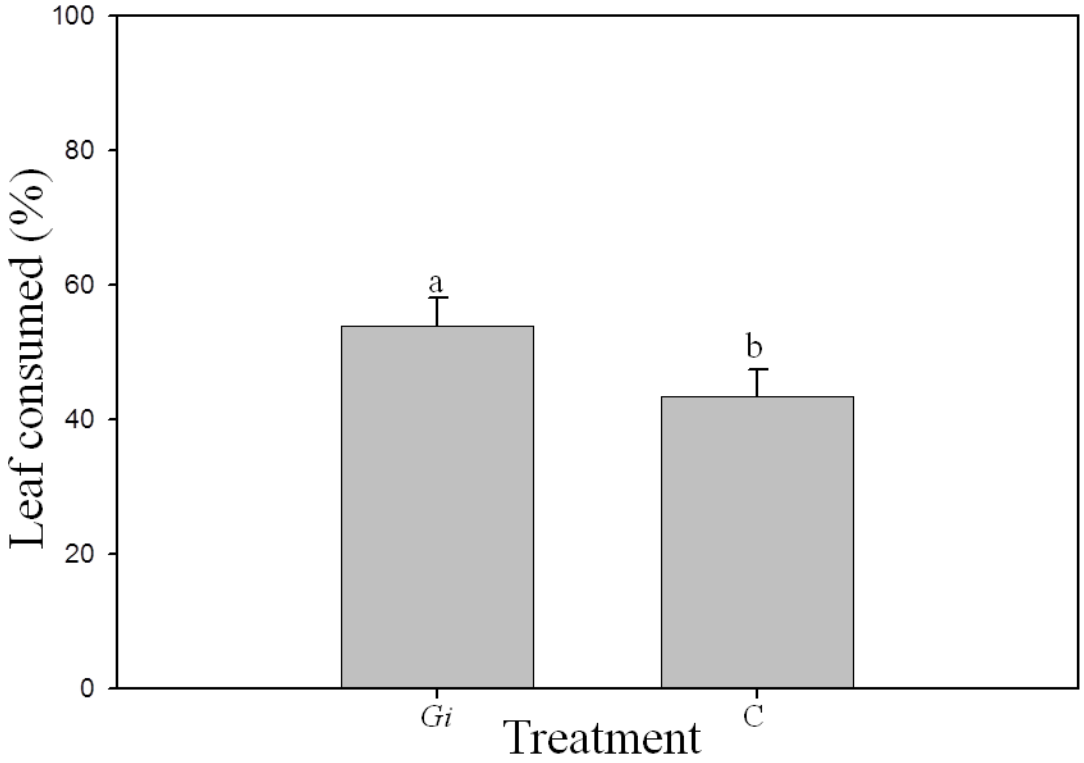

Fig. 3.35. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) - image analysis of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Percentage consumption was determined using Assess 2.2 Image Analysis Software for Plant Disease Quantification. Treatments: no-sorghum, no-mycorrhizae control (C); and sorghum colonized with *Glomus intraradices* (*Gi*). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.0898).

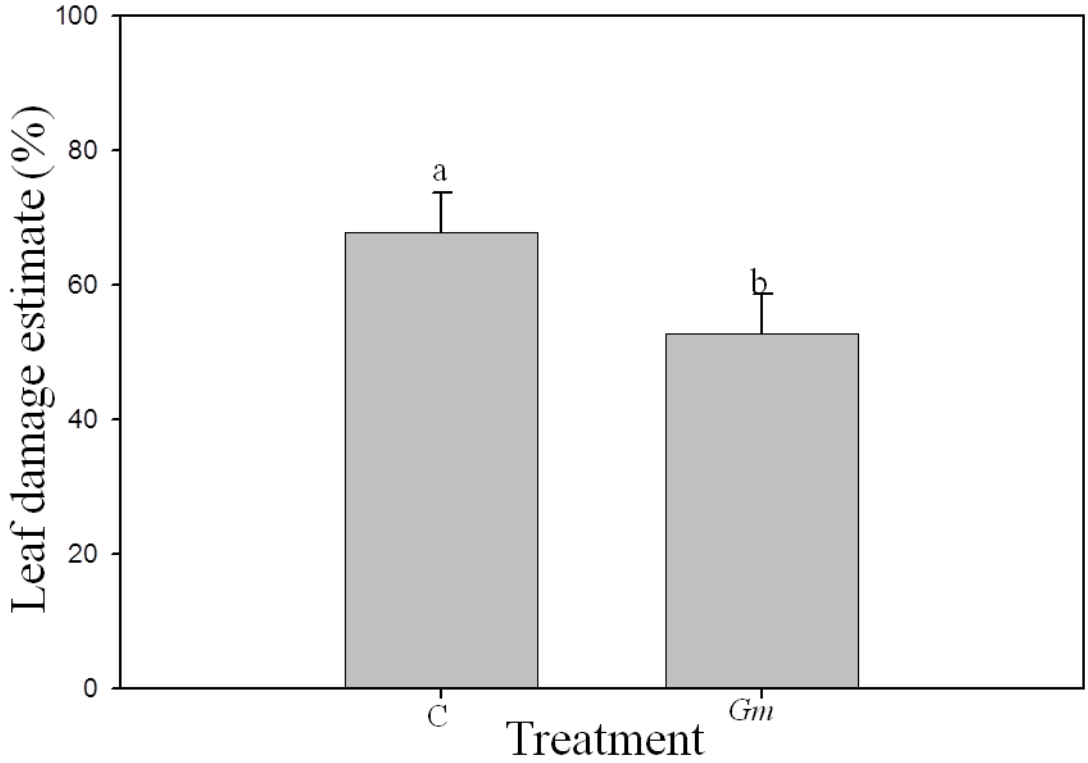

Fig. 3.36. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) – subjective estimate of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Damage estimates are the mean of two raters' subjective scores. Treatments: no-mycorrhizae, no-sorghum control (C); and sorghum colonized with *Gigaspora margarita* (*Gm*). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.0908).

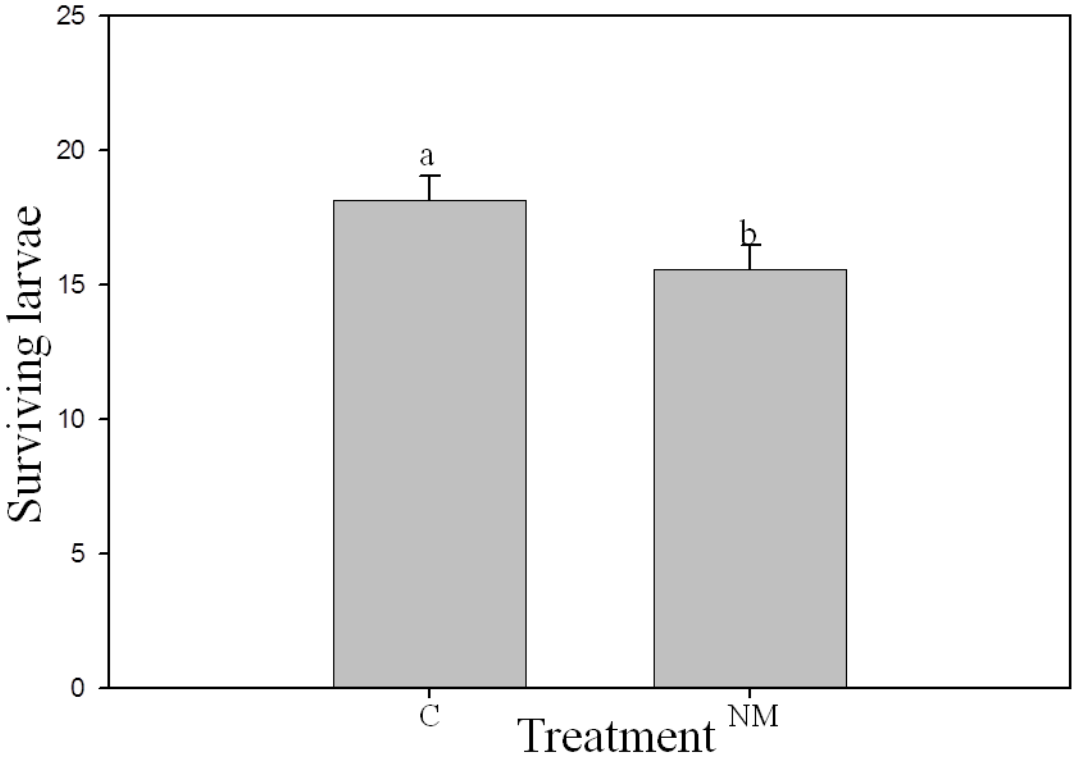

Fig. 3.37. Effect of mycorrhizae on fall armyworm (FAW) (Spodoptera frugiperda) feeding on wheat leaves (choice test) – surviving larvae. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Treatments: no- mycorrhizae, no sorghum control (C); and non- mycorrhizal sorghum (NM). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.0669).

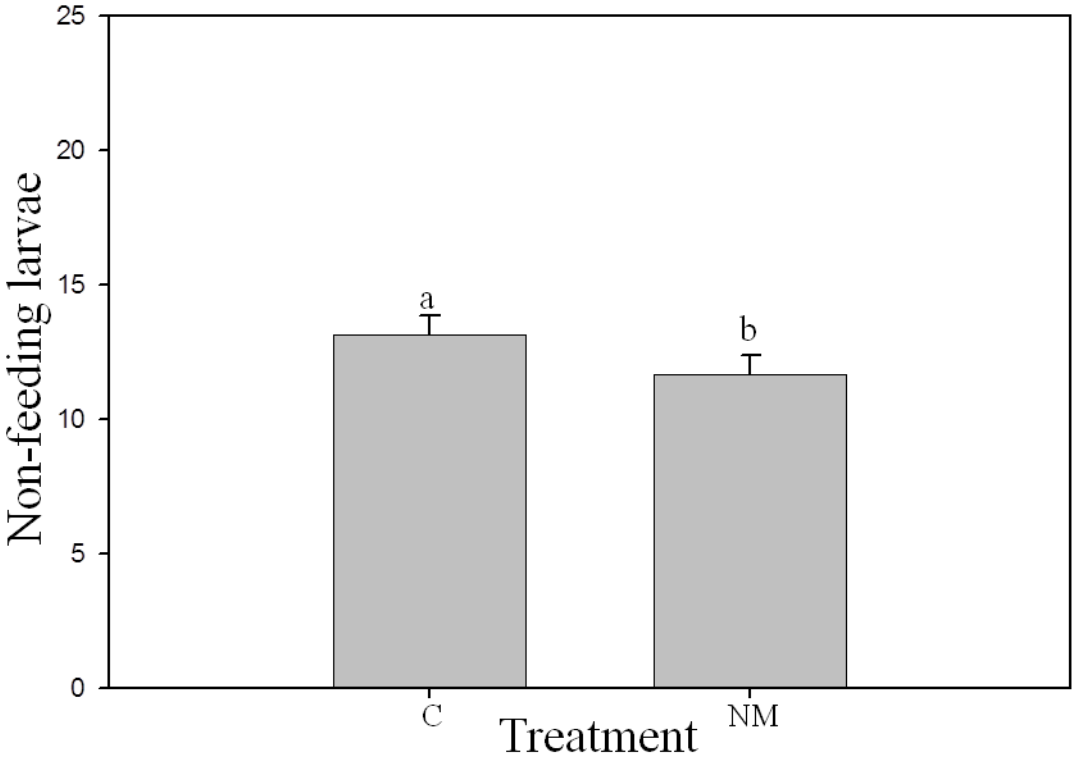

Fig. 3.38. Effect of mycorrhizae on fall armyworm (FAW) (Spodoptera frugiperda) feeding on wheat leaves (choice test) – non-feeding larvae. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Treatments: no-mycorrhizae, no-sorghum control (C); and non- mycorrhizal sorghum (NM). Bars with the same letter are not different according to an F-protected LSD (*P*= 0.0650).

No-choice experiment. In these experiments, the insect arena contained leaf segments from only one of the following treatments: C, *Gm*, *Gi*, or NM. Survival of

larvae was not different among treatments in either trial (*P*= 0.3610, Trial A; *P*= 0.7220, Trial B).

When control leaves were removed from the analysis, larval survival was not different between the mycorrhizal and non-mycorrhizal (NM) treatments (*P*= 0.3195, Trial A; *P*= 0.7639, Trial B). Treatment had no effect on subjective estimates of leaf damage caused by FAW larvae (*P*= 0.2491, Trial A; *P*= 0.4272, Trial B). When control leaves were removed from the analysis, there was no difference among treatments in either trial (*P*= 0.1607, Trial A; *P*= 0.2436, Trial B). There was no effect of treatments on percentage leaf consumed in either trial (*P* = 0.4551, Trial A; *P* = 0.7811, Trial B). When control leaves were excluded from the analysis, mycorrhizal and non-mycorrhizal (NM) treatments were not different (*P*= 0.7263, Trial A; *P*= 0.7011, Trial B).

When the image analysis estimates were converted to a published feeding scale (0 to 3) (Hardy et al., 1985), treatments were not different in either trial (*P*= 0.2601, Trial A; *P*= 0.6442, Trial B). When control leaves were excluded from the analysis in order to detect any difference between mycorrhizal and non-mycorrhizal (NM) leaves, there were no differences was among treatments (*P*= 0.3042, Trial A; *P*= 0.4941, Trial B).

3.5. Effect of mycorrhizae on seedling disease caused by *Bipolaris sorokiniana*

Mycorrhizal colonization levels of wheat seedlings used in disease assays are shown in Table 3.8.

Table 3.8. Arbuscular colonization (AC), vesicular colonization (VC), and hyphal colonization (HC) of 6-week-old wheat seedlings used in seedling disease assays. Treatments are inocula obtained from either sorghum plants colonized with *Gigaspora margarita* (*Gm*) or sorghum colonized with *Glomus intraradices* (*Gi*).

Treatment	Trial	AC (%)	VC (%)	HC (%)
Gm	A	0.015	.	0.89
Gi	A	0.001	0.005	0.97
Gm	B	0.004	.	0.066
Gi	B	.	.	.

No-mycorrhizae, no-sorghum control plants (C) were included in the experiments as a positive check for pathogensis but were eliminated from the statistical analysis. Mycorrhizal colonization of wheat seedlings by *Gm* or *Gi* had no effect on the number of surviving plants in either Trial A or Trial B (Fig. 3.39). For shoot height and weight, and disease rating, there was no effect of pathogen in either trial ($P \leq 0.05$).

Wheat seedlings colonized with *Gm* or *Gi* had greater shoot height than non-mycorrhizal plants (NM) in Trial A; there were no differences among the treatments in Trial B (Fig. 3.40). In Trial A, fresh shoot weight was greater in wheat plants colonized by *Gm* than in non-mycorrhizal plants (NM), but no difference was observed between *Gi* and non-mycorrhizal (NM) plants (Fig. 3.41). In Trial B, there were no differences among treatments in fresh shoot weight (Fig. 3.41). In Trial A, wheat colonized by *Gm* had greater dry shoot weight than non-mycorrhizal (NM) plants, but mycorrizal and non-mycorrhizal wheat seedling plants (NM) in trial B did not differ in dry shoot weight (Fig. 3.42).

No treatments differed in fresh root weight for either trial (Fig. 3.43). In Trial B, there was an effect of pathogen on the dry weight of wheat seedling roots; roots colonized by *Gi* and treated with water weighed less than roots colonized by *Gi* and inoculated with *Bs* spores (Fig. 3.44). The disease rating of NM plants was greater than *Gm* plants in Trial A, but there were no differences in Trial B (Fig. 3.45).

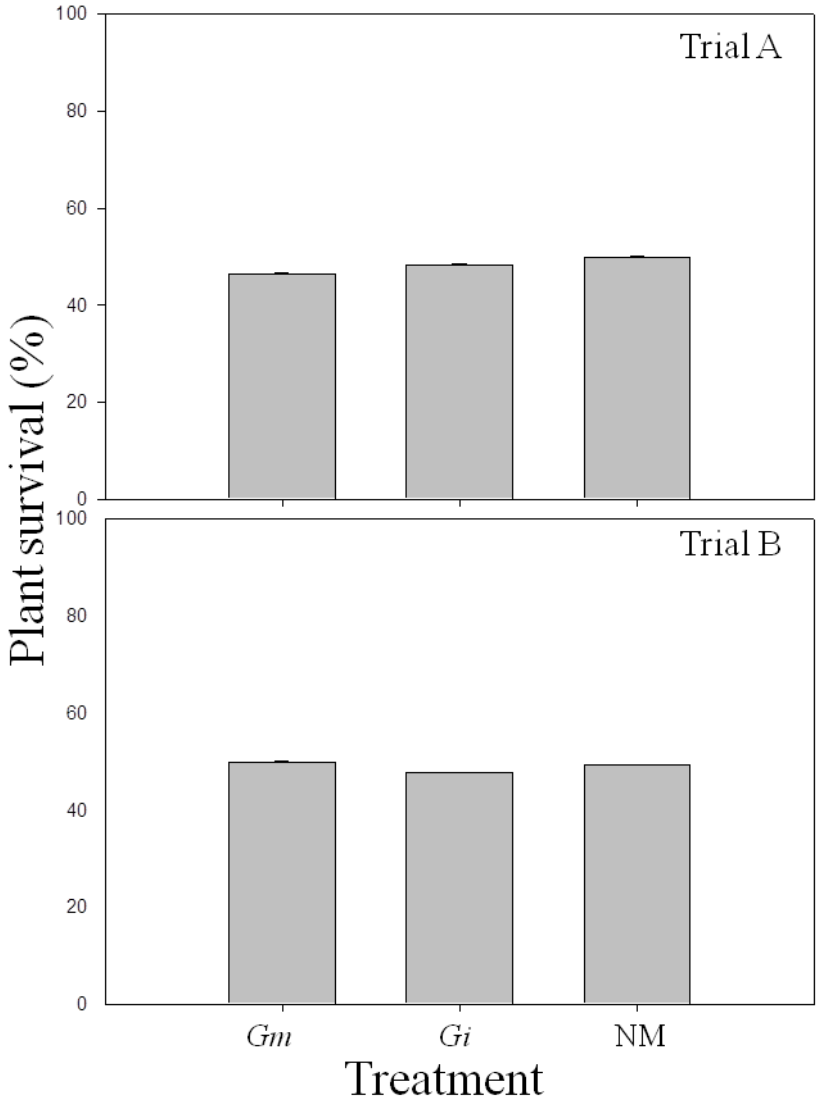

Fig. 3.39. Effect of mycorrhizae on plant survival (%) of wheat plants. Wheat seedlings were planted and harvested after 6 weeks in substrate containing sorghum with or without mycorrhizae. At 4 weeks, the aboveground portions of the wheat seedlings were sprayed with either water or a suspension of *Bipolaris sorokiniana* spores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.4448, Trial A; *P*= 0.2736, Trial B).

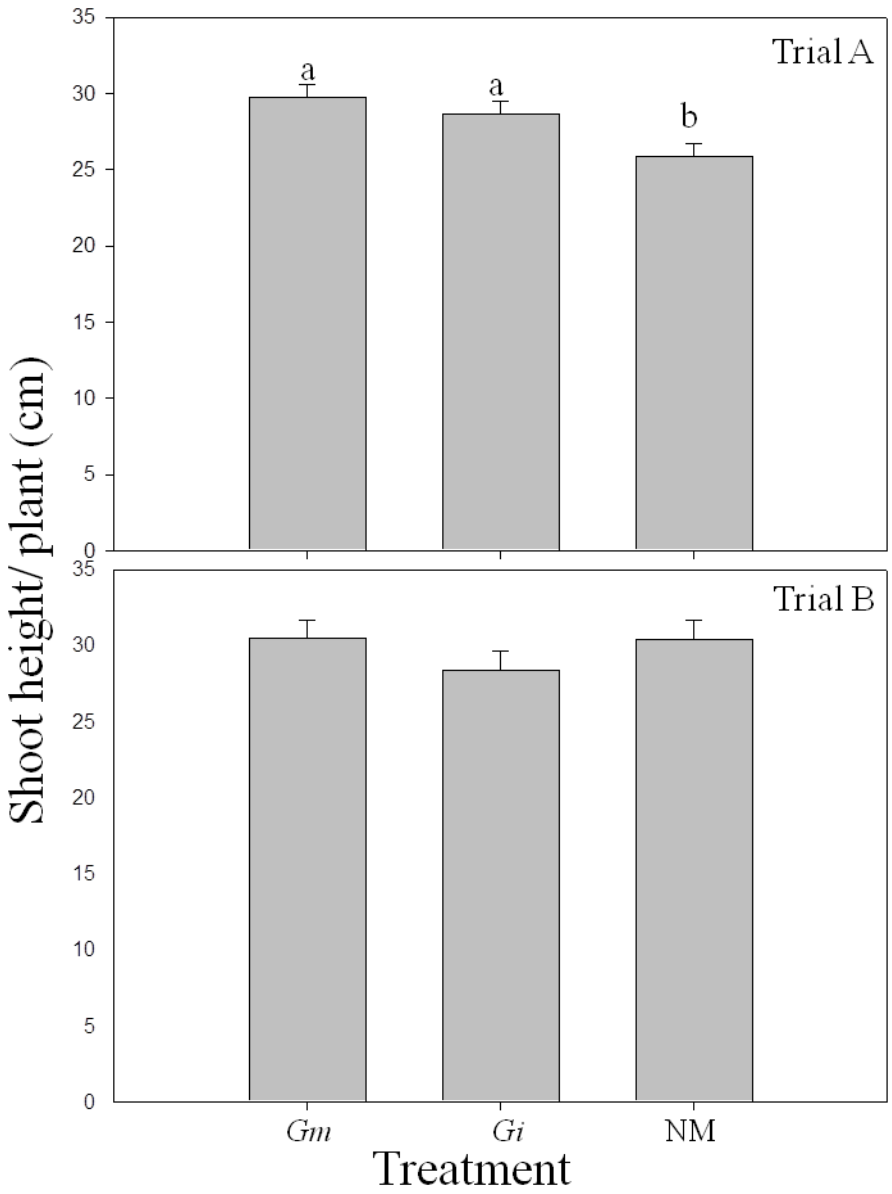

Fig. 3.40. Effect of mycorrhizae on shoot height (cm) of wheat plants. Wheat seedlings were planted and harvested after 6 weeks in substrate containing sorghum with or without mycorrhizae. At 4 weeks, the aboveground portions of the wheat seedlings were sprayed with either water or a suspension of *Bipolaris sorokiniana* spores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.0100, Trial A; *P*= 0.3791, Trial B).

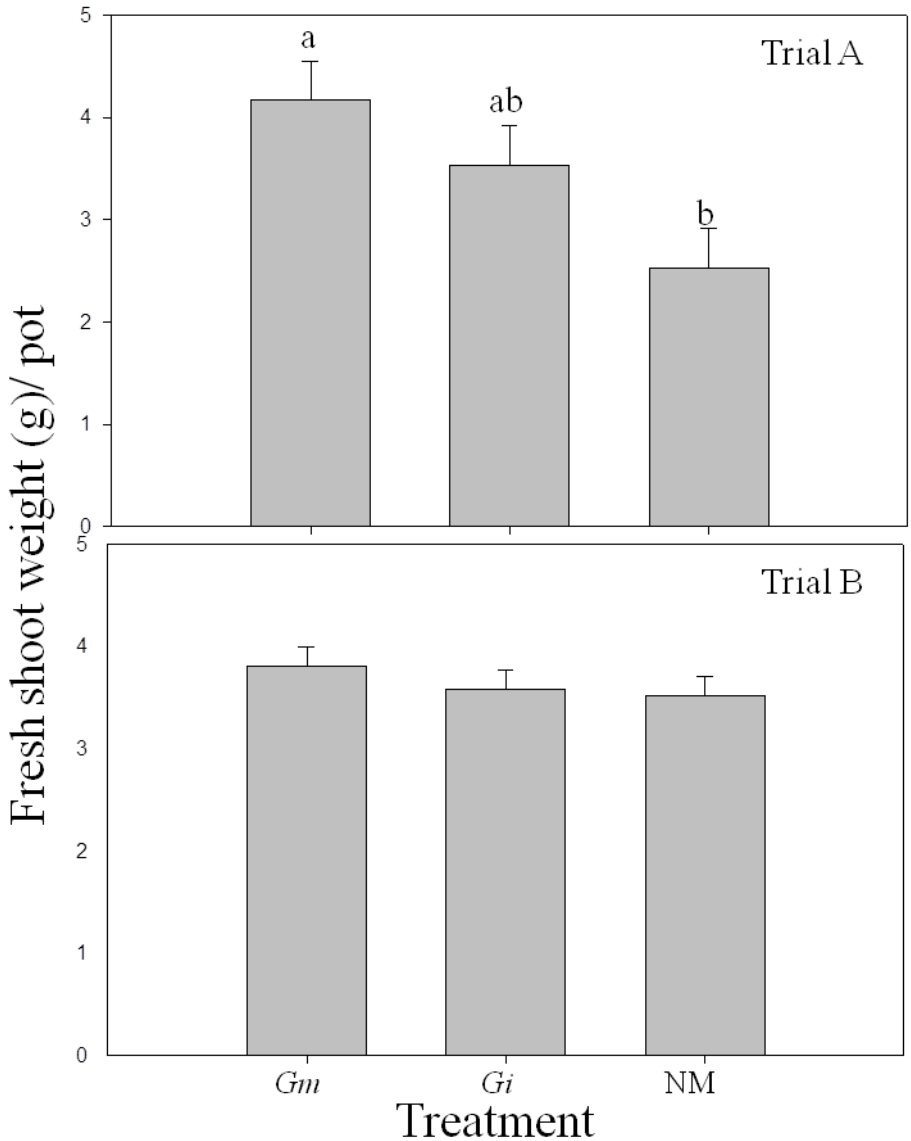

Fig. 3.41. Effect of mycorrhizae on fresh shoot weight (g) of wheat plants. Wheat seedlings were planted and harvested after 6 weeks in substrate containing sorghum with or without mycorrhizae. At 4 weeks, the aboveground portions of the wheat seedlings were sprayed with either water or a suspension of *Bipolaris sorokiniana* spores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.0250, Trial A; *P*= 0.5611, Trial B).

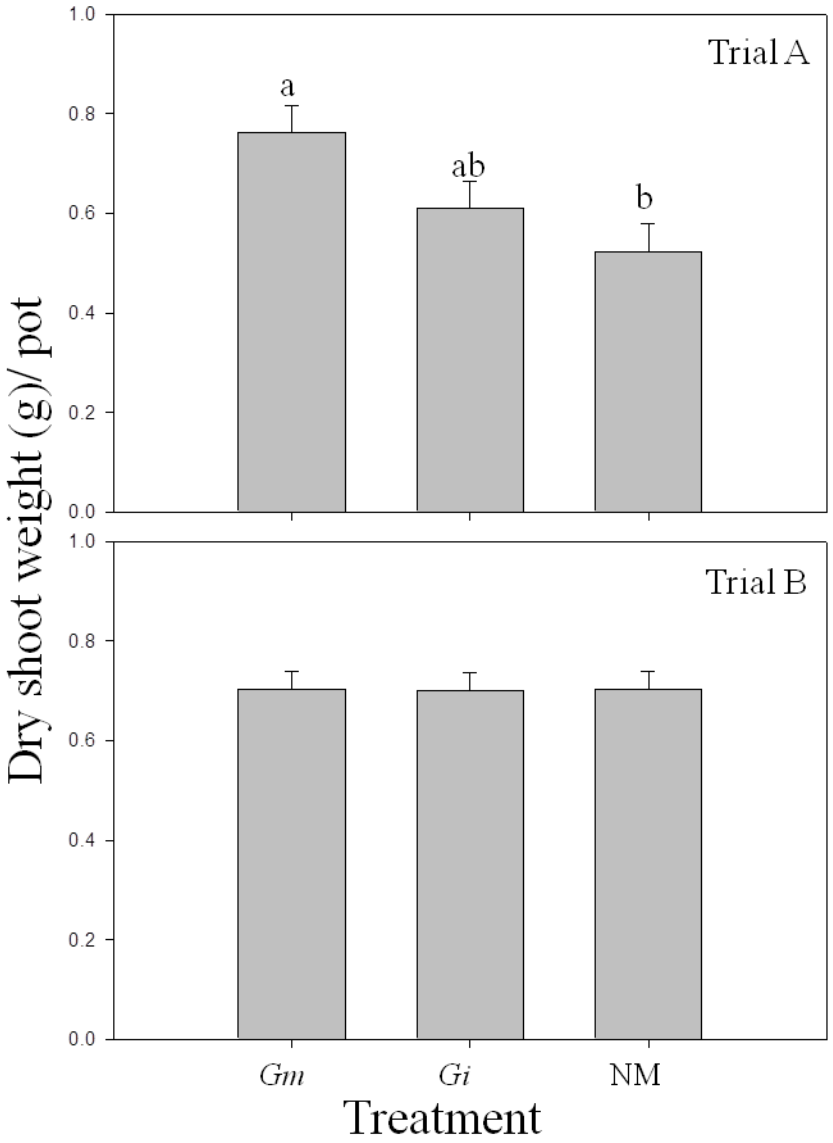

Fig. 3.42. Effect of mycorrhizae on dry shoot weight (g) of wheat plants. Wheat seedlings were planted and harvested after 6 weeks in substrate containing sorghum with or without mycorrhizae. At 4 weeks, the aboveground portions of the wheat seedlings were sprayed with either water or a suspension of *Bipolaris sorokiniana* spores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.0235, Trial A; *P*= 0.9957, Trial B).

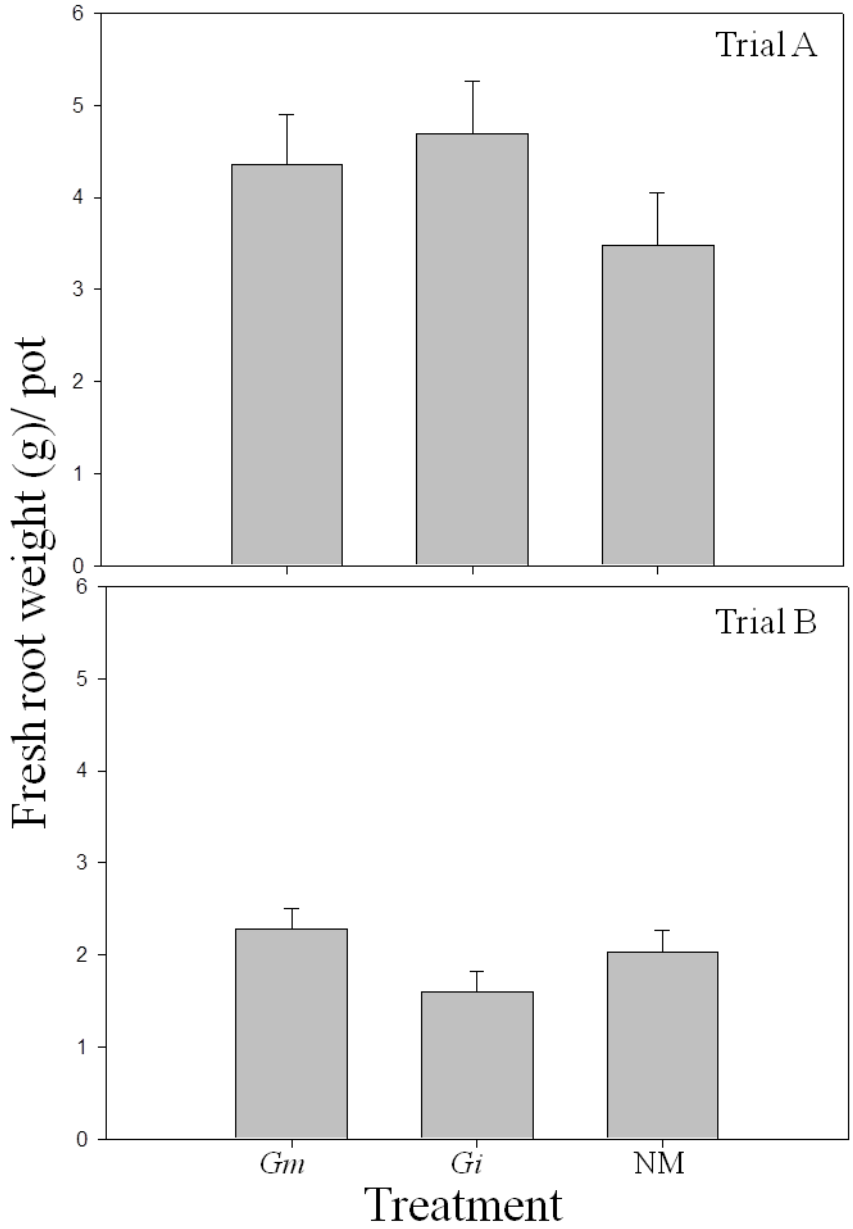

Fig. 3.43. Effect of mycorrhizae on fresh root weight (g) of wheat plants. Wheat seedlings were planted and harvested after 6 weeks in substrate containing sorghum with or without mycorrhizae. At 4 weeks, the aboveground portions of the wheat seedlings were sprayed with either water or a suspension of *Bipolaris sorokiniana* spores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.3196, Trial A; *P*= 0.0722, Trial B).

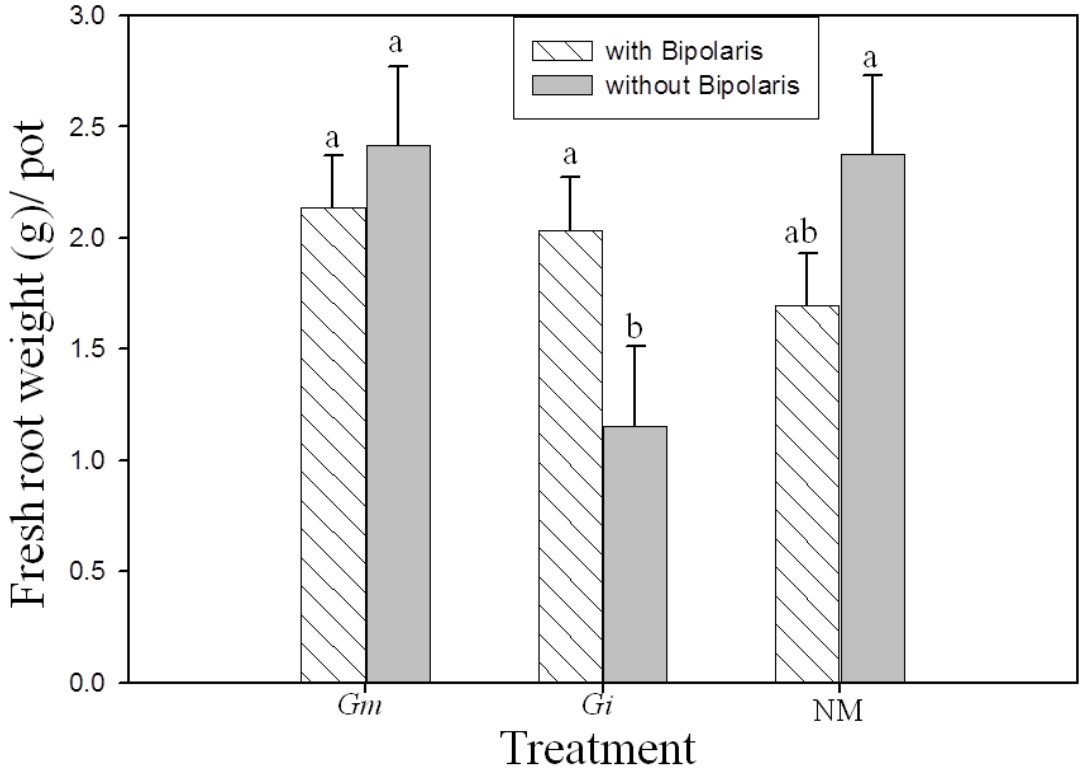

Fig. 3.44. Effect of mycorrhizae and pathogen on fresh root weight (g) of wheat plants. Wheat seedlings were planted and harvested after 6 weeks in substrate containing sorghum with or without mycorrhizae. At 4 weeks, the aboveground portions of the wheat seedlings were sprayed with either water or a suspension of *Bipolaris sorokiniana* spores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Bars with same letter are not different according to an F-protected LSD (*P*= 0.0317, Trial B).

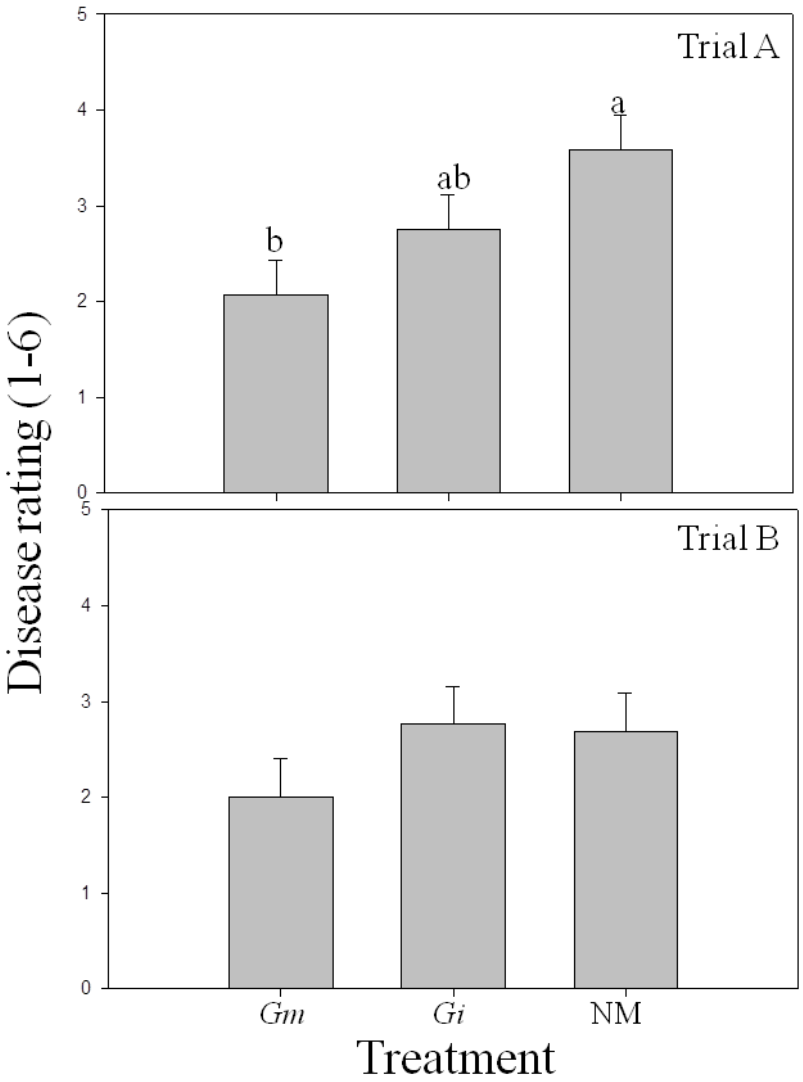

Fig. 3.45. Effect of mycorrhizae on disease rating (1-6) of wheat plants- subjective rating scale. Wheat seedlings were planted and harvested after 6 weeks in substrate containing sorghum with or without mycorrhizae. At 4 weeks, the aboveground portions of the wheat seedlings were sprayed with either water or a suspension of *Bipolaris sorokiniana* spores. Disease estimates are the mean of two subjective scores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). (Scores are based on a 0 to 6 scale where 0 = healthy, tillering plants with no lesions, and and 6 = stunting plants with large portions of necrotic tissue. Full explanation of the disease index scale can be found in Table 2.1 (Chapter 2). Within each trial, bars with the same letter or without letters are not different according to an F-protected LSD (*P*= 0.0290, Trial A; *P*= 0.3452, Trial B).

Chapter 4

Discussion

Mycorrhizal colonization levels of the 4-week-old seedlings were low throughout the research. The highest level (46%) of arbuscular colonization was in the *Gi* treatment of the sorghum allelopathy study; the highest hyphal colonization rate (80%) was in the *Gm* treatment in the same study. The lowest colonization rates (<0.1%) were obtained in the wheat seedlings used in the seedling disease assays. The method used in this research does not address the intensity of the colonization but only gives a positive or negative in each microscope view so it is possible that the low levels are not truly reflective of the actual colonization status of the plant. Low colonization levels are of concern, but in many studies there are no clear relationships between colonization level and physiological changes in the host. This has been documented best by the lack of a relationship between colonization levels and plant biomass production. In poor soils, colonization level is typically poor and not related to dry matter production (Clark, 1997). In a test of five durum wheat cultivars, there was no relationship between colonization level and productivity. One cultivar, 'Commander', had the highest colonization levels of five tested cultivars under low soil fertility conditions but developed poor colonization levels under medium fertility levels (Singh et al., 2012). In other studies, no correlation was found between mycorrhizal colonization level and wheat yield improvements (Ryan and Graham, 2002). In the *Bipolaris* experiments, contamination by root-inhabiting pathogens may have reduced the ability of the mycorrhizae to colonize the plants.

Allelopathy is the effect, either stimulatory or inhibitory, of one plant on another neighboring plant; allelochemicals are often released from the plant via root exudates or plant decomposition. Sorghum roots produce an array of detrimental allelochemicals; the most studied of these is the phenolic acid, sorgoleone (2-hydroxy-5-methoxy-3-[(8'Z, 11'Z)-8', 11', 14'-petadecatriene]-p-hydroquinone). Sorgoleone production is correlated

with significant yield decreases in subsequent crops (Roth et al., 2000; Benhammouda et al., 1995; Dayan et al., 2009; and Rasmussen et al., 1992). Wheat is particularly sensitive to sorghum allelopathy and sorgoleone (Roth et al., 2000). In this study, wheat plants that followed sorghum were typically smaller than control plants that did not follow sorghum. For example, in tests with the high sorgoleone sorghum x Sudangrass hybrid, plant heights and shoot weights in the *Gi*, *Gm*, and NM treatments were approximately 65% and 40%, respectively, of control plants that were not exposed to sorghum allelopathy.

We investigated the impact of sorghum on wheat and the role of arbuscular mycorrhizae fungi (AMF) in alleviating allelopathic effects. Mycorrhizal relationships are classified as neutral (no effect), positive, or negative, but in most studied systems, the impact is positive. The effect of mycorrhizae on plant growth was typically neutral in our allelopathy studies with *S. bicolor* when the no-sorghum, no-mycorrhizae controls were included in the analysis. The exception is the fresh weight of roots where the root weight in the *Gm* treatments was greater than in the *Gi*. When results were analyzed without control to better define the role of the mycorrhizae, the relationships between the NM and the mycorrhizal treatments did not change. Regardless of the mycorrhizae isolate, non-mycorrhizal and mycorrhizal wheat seedlings were not typically different in plant biomass. This differs from a previous report in which wheat colonized by *Gi* had greater yield in fields with low phosphorus level that had previously been planted with sorghum (Mohammad and Khan, 2004). However, although the propagation mix used in this study is low in phosphorous, higher amounts of phosphorous were used in the fertigation system for both the no-sorghum control and the NM treatments so phosphorous was not limited in the nonmycorrhizal treatments, and thus, increased phosphorus probably did not play a significant role in our study.

Growth and development of wheat seedlings grown in a substrate containing roots of a sorghum x Sudangrass hybrid known to produce high concentrations of sorgoleone (Dayan et al. 2009), showed similar patterns to that of wheat seedlings planted in nonhybrid sorghum (*S. bicolor*). Control plants were clearly taller and more robust than mycorrhizal and non-mycorrhizal plants. Lack of effect of sorghum allelopathy on wheat

seedling germination has been reported for wheat seed exposed to sorghum hybrid extract (Benhammouda et al., 1995) and is consistent with findings in this study. When mycorrhizae were cultured on the hybrid, inconsistent results were found between NM and mycorrhizal plants. Although shoot height was greater in wheat seedlings colonized with *Gi* than in NM, the NM plants had greater fresh shoot weight and dry root weight compared to *Gi* seedlings. Plant biomass of seedlings colonized by *Gm* was not different from the two other treatments, despite the fact that plant colonization level by *Gm* was less than that of *Gi.* Mycorrhizal colonization levels were less in the study using the hybrid sorghum; hyphal colonization levels for *Gm* were approximately 1.5-times greater than those for Gi in the *S. bicolor* study but were 1.5 lower in the study with the hybrid

In addition to reduced growth, plants with to sorghum allelopathy are often yellow in color due to the effect of the sorgoleone on chlorophyll. Since activation of chlorophyll pigments allows the conversion of light energy into chemical energy via series of electron transfers, treatment with sorgoleone results in a reduction in net photosynthesis. The primary effect of sorgoleone is the inhibition of electron transport in photosystem II (PS II). Sorgoleone does not affect photosystem I (PS I) (Nimbal et al. 1996). Photosystem I consists largely of Chlorophyll A molecules and contains little Chlorophyll B; whereas PSII contains both Chlorophyll A and B. In this study, chlorophyll B concentration in *Gi*-colonized wheat leaves was greater than other treatments. Chlorophyll A concentration in control (C) and *Gi* wheat plants was higher than in *Gm*-colonized plants. The ratio of Chlorophyll A to Chlorophyll B (A/B ratio) was greater in control and non-mycorrhizal (NM) plants than in mycorrhizal plants in experiments using *S. bicolor*. In experiments with the high sorgoleone hybrid, the A/B ratio was reduced in NM compared to all other treatments. In the allelopathy studies, total chlorophylls for *Gm* treatments were less than the no-sorghum control; however, in allelopathy studies with the hybrid, total chlorophyll in *Gi* treatments was lower than either control or *Gm*, and control and *Gm* were not different. In leaves of pistachio plants (*Pistacia vera* L.) colonized with *Glomus intraradices* (*Gi*) or *G. mosseae;* mycorrhizal plants had greater Chl A, Chl B and carotenoid concentrations than non-mycorrhizal plants (Bagheri et al., 2011).

Plants colonized by AMF have great benefits such as improved nutrient uptake (Smith and Read, 2008), increased water absorption (Augé, 2001), and altered host physiology to induce plant host defense systems by stimulating various genes that encode anti-herbivore compounds (e.g., jasmonic and salicylic acids), and plant isoflavonoid compounds in mycorrhizal roots that act as antifungal compounds (Morandi et al., 1984; Abdel-Fattah et al., 2011). Mycorrhizal colonization induces activation of host defense systems. Insect herbivory may be reduced as a result of the production of antifeedant compounds in shoots (Pozo and Augilar, 2007). In general, mycorrhizal colonization is reported to have positive effects (e.g., increased larval weight, and survival rate) on phloem-feeding insects such as aphids, but determintal effects (e.g., reduced larval growth) are seen on chewing insects (e.g., beetle) (Gange et al., 2002). The proposed mechanism is that mycorrhizal association improves plant nutrient uptake, and thus improves food quality for the phloem-feeding insects. Narrowleaf plantain (*Plantago lanceolata* L.) colonized with *G. intraradices* supported greater numbers of two aphid species, *Myzus ascalonicus* and *M. persicae* **(**Gange et al., 1999).

Aphids reared on mycorrhizal plants produced more offspring, and had greater weight than aphids reared on non-colonized plants **(**Gange et al., 1999). However, in two natural infestations in our greenhouses, non-mycorrhizal wheat seedlings grown in the presence of sorghum roots attracted more bird cherry-oat aphids than control or mycorrhizal plants. To determine whether or not mycorrhizal colonization of wheat seedlings could increase resistance against insects, both choice and no-choice tests were conducted. Wheat plants colonized with *Gi* had fewer aphids than non-mycorrhizal plants in the no-choice experiment; however, aphid numbers were not different in *Gm*-colonized wheat plants when compared to either *Gi* or NM plants. We hypothesized that volatile compounds were emitted from non-mycorrhizal plants that attracted the hovering aphids or that compounds were emitted from mycorrhizal plants that deterred insects (Fig. 3.25). In the choice test, slight differences in the volatile profiles were detected by the GC-MS analysis, but numbers of aphids on mycorrhizal and non-mycorrhizal plants were not different. Mycorrhizal plants colonized by *Gm* emitted larger amounts of butyronitrite, 2-

ethylhexyl ester, and benzoic acid than their non-colonized counterparts (NM). Plant host resists herbivory invasion via producing several anti-herbivory compounds such as salicylic acid (SA) and jasmonic acid (JA) (Li et al., 2002). Salicylic acid is a hydroxylated benzoic acid, so more SA might have been produced in the mycorrhizal plants than in the nonmycorrhizal plants in our study (Me′traux, 2002). Salicylic acid is a known repllent compound of *R. padi*; high concentrations of SA are associated with migration of *R. padi* from its bird cherry host to the grass hosts (Pettersson et al., 1994).

Due to the collapse of our *R. padi* colony, fall armyworm was selected for further studies because it is: 1) commonly used in host-herbivory experiments; 2) commercially available; and 3) a good model for chewing-mouth type of insects. Leaves from mycorrhizal plants, in particular *Gi*, inoculated with fall armyworm larvae were consumed less than the control wheat leaves in Trial A of the choice experiements. Other variables such as leaf consumed, damage rating, and surviving larvae were not different among treatments, irrespective of mycorrhizal isolates. Although fall armyworm has been widely used in plant-endophyte-herbivor interactions, it was not an appropriate model for testing mycorrhizal-host-herbivor interaction under our experimental conditions.

Two *Bipolaris sorokiniana* isolates, previously isolated from Wt 65 and Alamo switchgrass by Vu et al., 2011, inoculated on mycorrhizal or non-mycorrhizal wheat seedlings. In two experiments, there were no differences in growth or disease rating between inoculated wheat seedlings with *Bs* and inoculated wheat seedlings with sterile water (control). There is a high degree of variability in aggressiveness of *Bs* isolates based on pathogen genetic variation, plant phenotype, and environmental conditions. The effects of environmental are reduced since the present experiments were done in controlled condition (i.e., growth chamber). We reasoned the low virulence of *Bs* is caused by one of the following factors: 1) inadequate spraying to cover all plants; and 2) insufficient concentration of *Bs* spores to induce disease. Moreover, our cultures were originally obtained from switchgrass leaves, and a study on wheat infected with *Bs* showed that the probability of culture originated from wheat roots to induce lesion were

higher than culture from wheat leaves (Duveiller, and Garcia, 2000). In barley plants, there were differences in the degree of virulence in 22 isolates of *Bs* were reported in North Carolina (Valjavec, and Steffenson, 1997). Their finding can lead to the hypothesis that our *Bs* isolates are not virulent on wheat seedlings. In our studies with *Bipolaris*, wheat seedlings colonized by *Gm* displayed low disease severity caused by *Bipolaris* species compared to non-mycorrhizal (NM) seedlings. Furthermore, *Gi*-colonized seedlings did not differ from *Gm* or non-mycorrhizal seedlings. Both mycorrhizal wheat seedlings were greater in shoot height than in non-mycorrhizal seedlings. The *Gi*- seedlings treated with *Bipolaris* species had greater root weight than untreated *Gi*- seedling. There was no effect of *Bipolaris* on either *Gm* or non-mycorrhizal (NM) seedlings. Although mycorrhizae application on barley plants decreased *B. sorokiniana* transmission from roots to the aboveground (Sjöberg et al., 2007), the current study data showed no impact of mycorrhiza on wheat seedling inoculated with or without *Bipolaris* species.

Chapter 5

Summary

Our experiments demonstrate that planting wheat seedlings on substrate containing sorghum roots reduce wheat growth parameters (e.g., height and weight) in comparison to control wheat seedlings (no-mycorrhizae, no-sorghum). Although sorghum is grown as cereal and cover crop, its allelopathic trait can be disadvantageous especially if the following crop, like wheat, is susceptible to sorghum allelopathy. Arbuscular mycorrhizal fungi are benefical microorganisms that provide their host plant with mineral uptake from the rhizosphere and increase the colonized host resistance against pathogen attack. The two mycorrhizal fungi (*Gigaspora margarita* and *Glomus intraradices*) that were used in these studies successfully colonized wheat roots with similar colonization levels. Colonization of wheat seedlings with AM fungi did not alleviate the allelopathic effect of sorghum as we anticipated, however, in some trials the effect of sorghum on mycorrhizal wheat was less than on non-mycorrhizal wheat seedlings. Also, wheat seedlings that were planted in media contained the other sorghum variety (Sorghum x Sudangrass hybrid) showed similar responses to the ones observed on *S. bicolor*. Dual cultures of mycorrhizae are typically used to ensure good colonization of the host. We believe that use of a dual culture rather than a single isolate of AMF might have ensured better colonization of the wheat, and alterations in wheat physiology may have been more pronounced.

The question of the impact of AMF colonization on seedling disease caused by *Bipolaris sororkinina* remains unanswered because there were no significant effects of the pathogen in these experiments. Virulence of the isolates used in this study was not determined on wheat before experimentation, and disease severity was typically low and highly variable. Use of a *Bipolaris* isolate known to cause significant damage to wheat may reveal a positive impact of mycorrhizae on disease.

On the other hand, mycorrhizal plants, particularly plants colonized with *Gi*, were less attractive to aphid than non-mycorrhizal plants. The consumption rate of wheat

leaves colonized with *Gi* by fall armyworm larvae was less than the other treatments. Thus, the mycorrhizal isolate *Gi* is better option than *Gm* if the purpose to insecrease the host tolerance against herbivory attack. Mycorrhizal application can be a usefull tool to reduce the damage that caused by herbivory attack.

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VITA

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Appendices

Appendix 1. Statistical values.

Table A.1. Statistical value for experiments on 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*).

Propagation Host	Trial	Plant parameter (%)	Control [a] (+/-)	*P*-value	F-value	DF
S. bicolor	A	Fresh shoot weight	+	0.0001	31.24	3
S. bicolor	A	Fresh shoot weight	-	0.4018	0.97	2
S. bicolor	A	Fresh root weight	+	0.0001	39.39	3
S. bicolor	A	Fresh root weight	-	0.0193	5.31	2
S. bicolor	A	Dry root weight	+	0.0001	25.69	3
S. bicolor	A	Dry root weight	-	0.0116	6.24	2
S. bicolor	A	Stem diameter	-	0.0009	1.85	3
S. bicolor	A	Stem diameter	-	0.0905	2.87	2
S. bicolor	A	Chl a	+	0.0052	5.67	3
S. bicolor	A	Chl b	+	0.0077	5.20	3
S. bicolor	B	Fresh shoot weight	+	0.0001	19.31	3
S. bicolor	B	Fresh shoot weight	-	0.5008	0.73	2
S. bicolor	B	Fresh root weight	+	0.0006	8.60	3
S. bicolor	B	Fresh root weight	-	0.1140	2.55	2
S. bicolor	B	Dry root weight	-	0.1266	2.40	2

(+) = Analysis includes the no-mycorrhize, no-sorghum (control) treatment;
(-) = analysis without control (C).

Table A.2. Statistical values for experiments on 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained a Sorghum x Sudangrass hybrid.

Propagation Host	Trial	Plant parameter	Control [a] (+/-)	*P*-value	F-value	DF
Hybrid	A	Shoot height	+	0.0001	32.1	3
Hybrid	A	Shoot height	-	0.0275	12.1	2
Hybrid	A	Fresh shoot weight	+	0.0001	47.51	3
Hybrid	A	Fresh shoot weight	-	0.0315	4.47	2
Hybrid	A	Fresh root weight	+	0.4728	0.87	3
Hybrid	A	Dry root weight	+	0.0092	4.98	3
Hybrid	A	Dry root weight	-	0.0137	5.92	2
Hybrid	A	Stem diameter	-	0.0009	8.17	3
Hybrid	A	Shoot height	+	0.0001	71.52	3
Hybrid	A	Chl a	+	0.0072	5.28	3
Hybrid	A	Chl b	+	0.1603	1.90	3
Hybrid	A	Total Chl	+	0.0359	3.42	3
Hybrid	B	Shoot weight	+	0.0001	38.15	3
Hybrid	B	Fresh root weight	+	0.3249	1.23	3
Hybrid	B	Dry root weight	+	0.0092	5.16	3
Hybrid	B	Shoot height	+	0.0001	33.21	3
Hybrid	B	Shoot height	-	0.3432	20.3	2
Hybrid	B	Fresh shoot weight	+	0.0001	33.4	3
Hybrid	B	Fresh shoot weight	-	0.0001	12.4	2

(+) = Analysis includes the no-mycorrhize, no-sorghum (control) treatment;
(-) = analysis without control (C).

Table A.3. Statistical values for experiments on 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*) and infested with bird cherry-oat aphid (*Rhopalosiphum padi*).

Test type	Trial	Plant parameter (%)	***P***-value	F-value	DF
Choice test	A	Plant survival	0.1070	2.82	2
Choice test	A	Aphid number	0.2584	1.55	2
No-choice	A	Plant survival	0.2689	1.86	2
No-choice	A	Plant height	0.3435	1.15	2
No-choice	A	Fresh shoot weight	0.0171	13.30	2
No-choice	A	Aphid number	0.0912	4.62	2
No-choice	B	Aphid number	0.0955	4.47	2

Table A.4. Statistical values for experiments on leaves (Trial A) collected from 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*). Leaves were used to feed fall armyworm (*Spodoptera frugiperda*) larvae.

Experiment type	Plant parameter (%)	Control [a] (+/-)	*P*-value	F-value	DF
Choice	Leaf damage estimate	+	0.0303	3.61	3
Choice	Damage rating	+	0.2527	1.47	3
Choice	Leaf consumed	+	0.2582	1.44	3
Choice	Leaf damage estimate	-	0.2975	1.32	2
Choice	Damage rating	-	0.0638	3.37	2
Choice	Leaf consumed	-	0.1045	2.67	2
No-choice	Larvae surviving	+	0.3610	1.13	3
No-choice	Leaf damage estimate	+	0.2491	1.48	3
No-choice	Damage rating	+	0.2601	1.44	3
No-choice	Leaf consumed	+	0.4551	0.91	3
No-choice	Larvae surviving	-	0.3195	1.24	2
No-choice	Leaf damage estimate	-	0.1607	2.09	2
No-choice	Damage rating	-	0.3042	1.30	2
No-choice	Leaf consumed	-	0.7263	0.33	2

(+) = Analysis includes the no-mycorrhize, no-sorghum (control) treatment, and (-) = analysis without control (C).

Table A.5. Statistical values for experiments on leaves (Trial B) collected from 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*). Leaves were used to feed fall armyworm (*Spodoptera frugiperda*) larvae.

Experiment type	Plant parameter (%)	Control [a] (+/-)	*P*-value	F-value	DF
Choice	Leaf damage estimate	+	0.2982	1.31	3
Choice	Damage rating	+	0.4421	0.93	3
Choice	Leaf consumed	+	0.2677	1.41	3
Choice	Leaf damage estimate	-	0.1555	2.13	2
Choice	Damage rating	-	0.2652	1.46	2
Choice	Leaf consumed	-	0.1579	2.11	2
No-choice	Larvae surviving	+	0.7220	0.45	3
No-choice	Leaf damage estimate	+	0.4272	0.97	3
No-choice	Damage rating	+	0.6442	0.57	3
No-choice	Leaf consumed	+	0.7811	0.36	3
No-choice	Larvae surviving	-	0.7639	0.27	2
No-choice	Leaf damage estimate	-	0.2436	1.58	2
No-choice	Damage rating	-	0.4941	0.74	2
No-choice	Leaf consumed	-	0.7011	0.37	2

(+) = Analysis includes the no-mycorrhize, no-sorghum (control) treatment;
(-) = analysis without control (C).

Table A.6. Statistical values for choice tests (pairwise comparisons) on the numbers of fall armyworm (*Spodoptera frugiperda*) larvae feeding on leaves collected from 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*). Leaves were harvested at the same time as the leaves in the choice tests (all treatments) shown in Table A.6.

Trt 1	Trt 2	Plant parameter (%)	***P***-value	F-value	DF
Control	NM	Feeding larvae	0.8136	0.06	14
Control	NM	Non-feeding larvae	0.0650	4.01	14
Control	NM	Surviving larvae	0.0669	3.95	14
Gm	*Gi*	Feeding larvae	0.8849	0.02	14
Gm	*Gi*	Non-feeding larvae	0.5206	0.43	14
Gm	*Gi*	Surviving larvae	0.8578	0.03	14
Control	*Gm*	Feeding larvae	0.2462	1.47	14
Control	*Gm*	Non-feeding larvae	0.3302	1.02	14
Control	*Gm*	Surviving larvae	0.4226	0.68	14
Control	*Gi*	Feeding larvae	0.3577	0.90	14
Control	*Gi*	Non-feeding larvae	0.1502	2.32	14
Control	*Gi*	Surviving larvae	0.5870	0.31	14
NM	*Gm*	Feeding larvae	0.3118	1.10	14
NM	*Gm*	Non-feeding larvae	0.3315	1.01	14
NM	*Gm*	Surviving larvae	0.2390	1.51	14
NM	*Gi*	Feeding larvae	0.3862	0.80	14
NM	*Gi*	Non-feeding larvae	0.8854	0.02	14
NM	*Gi*	Surviving larvae	0.1949	1.85	14

Table A.7. Statistical values for choice tests (pairwise comparisons) on feeding estimates of fall armyworm (*Spodoptera frugiperda*) larvae feeding on leaves collected from 4-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*). Leaves were harvested at the same time as the leaves in the choice tests (all treatments) shown in Table A.6.

Trt 1	Trt 2	Plant parameter (%)	***P***-value	F-value	DF
Control	NM	Leaf damage estimate	0.7197	0.13	14
Control	NM	Damage rating	0.8062	0.06	14
Control	NM	Leaf comsumed	0.9346	0.01	14
Gm	*Gi*	Leaf damage estimate	0.9115	0.01	14
Gm	*Gi*	Damage rating	0.1038	3.03	14
Gm	*Gi*	Leaf comsumed	0.3972	0.76	14
Control	*Gm*	Leaf damage estimate	0.0908	3.30	14
Control	*Gm*	Damage rating	1.000	0	14
Control	*Gm*	Leaf comsumed	0.5700	0.34	14
Control	*Gi*	Leaf damage estimate	0.0529	4.47	14
Control	*Gi*	Damage rating	0.1755	2.04	14
Control	*Gi*	Leaf comsumed	0.0898	3.32	14
NM	*Gm*	Leaf damage estimate	0.1877	1.92	14
NM	*Gm*	Damage rating	0.7889	0.07	14
NM	*Gm*	Leaf comsumed	0.5631	0.35	14
NM	*Gi*	Leaf damage estimate	0.1468	2.36	14
NM	*Gi*	Damage rating	0.1362	2.50	14
NM	*Gi*	Leaf comsumed	0.1414	2.43	14

Table A.8. Statistical values for experiments on 6-week-old wheat seedlings colonized by mycorrhizae, previously grown in substrate that contained sorghum (*Sorghum bicolor*), and inoculated with or without *Bipolaris sorokiniana*.

Propagation Host	Trial	Plant parameter (%)	*P*-value	F-value	DF
S. bicolor	A	Plant survival	0.4448	0.85	2
S. bicolor	A	Disease rating	0.0290	4.45	2
S. bicolor	A	Shoot height	0.0100	6.23	2
S. bicolor	A	Fresh shoot weight	0.0250	4.69	2
S. bicolor	A	Dry shoot weight	0.0235	4.79	2
S. bicolor	A	Fresh root weight	0.3196	1.23	2
S. bicolor	B	Plant survival	0.2736	1.39	2
S. bicolor	B	Disease rating	0.3452	1.13	2
S. bicolor	B	Shoot height	0.3791	1.02	2
S. bicolor	B	Fresh shoot weight	0.5611	0.60	2
S. bicolor	B	Dry shoot weight	0.9957	0.00	2
S. bicolor	B	Fresh root weight	0.0722	3.05	2
S. bicolor	B	Fresh root weight / pathogen	0.0317	4.21	2

(+) = Analysis includes the no-mycorrhize, no-sorghum (control) treatment;
(-) = analysis without control (C).

Appendix 2. Effects of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test).

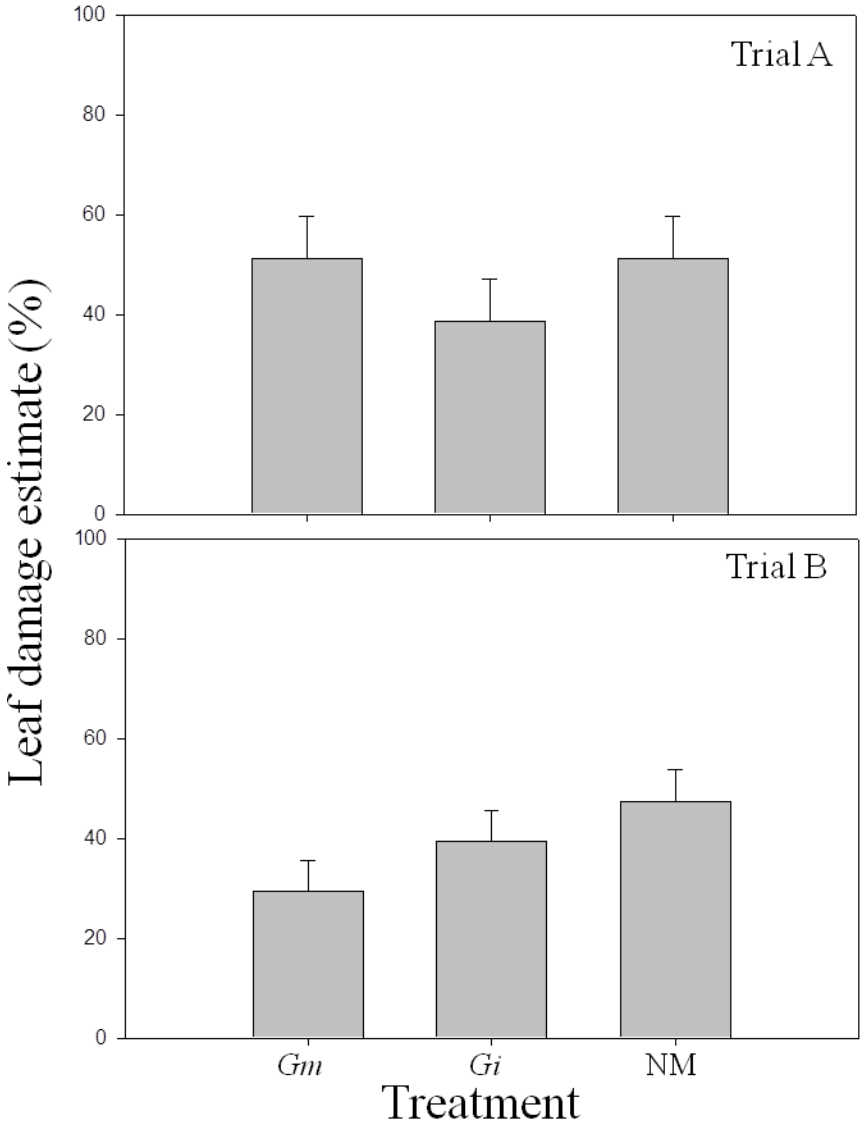

Fig. A.1. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) - subjective estimate of leaf damage (without control). Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; the arena contained all treatments. Excised leaves were obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Damage estimates are the mean of two raters' subjective scores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.2975, Trial A), (*P*= 0.1555, Trial B).

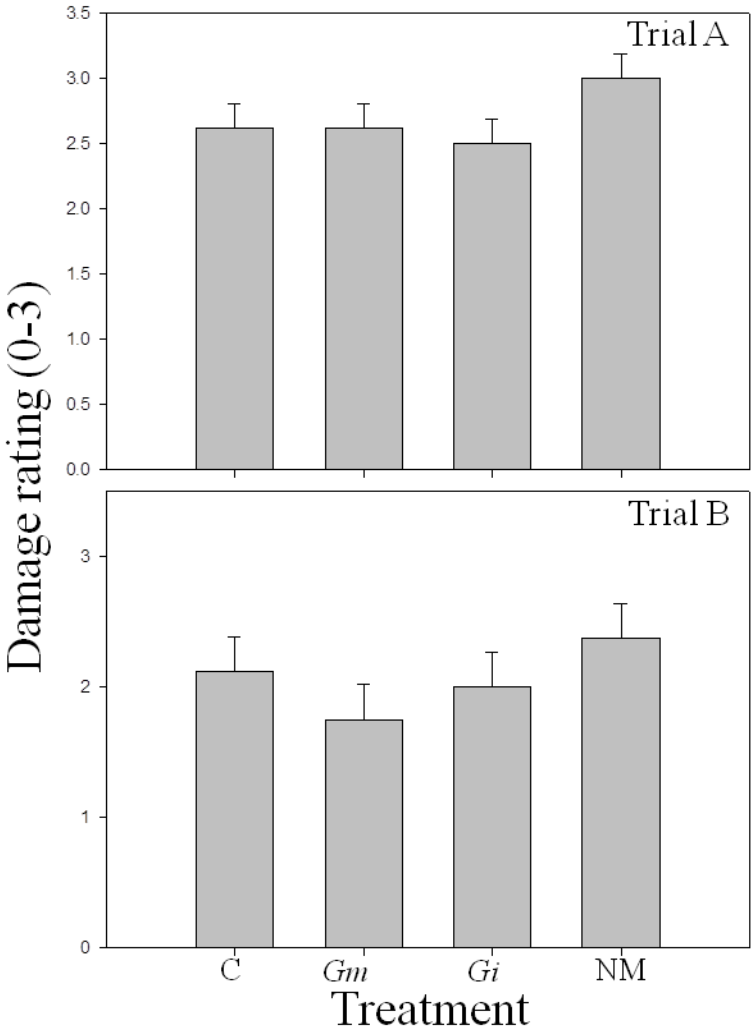

Fig. A.2. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) –rating scale. Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; the arena contained all treatments. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Image analysis damage estimate (%) was converted to the damage rating scale (0 – 3) developed by (Hardy et al., 1985). Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.252, Trial A; *P*= 0.442, Trial B).

Appendix 3. **Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugi*perda) feeding on wheat leaves (choice test/ comparsion test).**

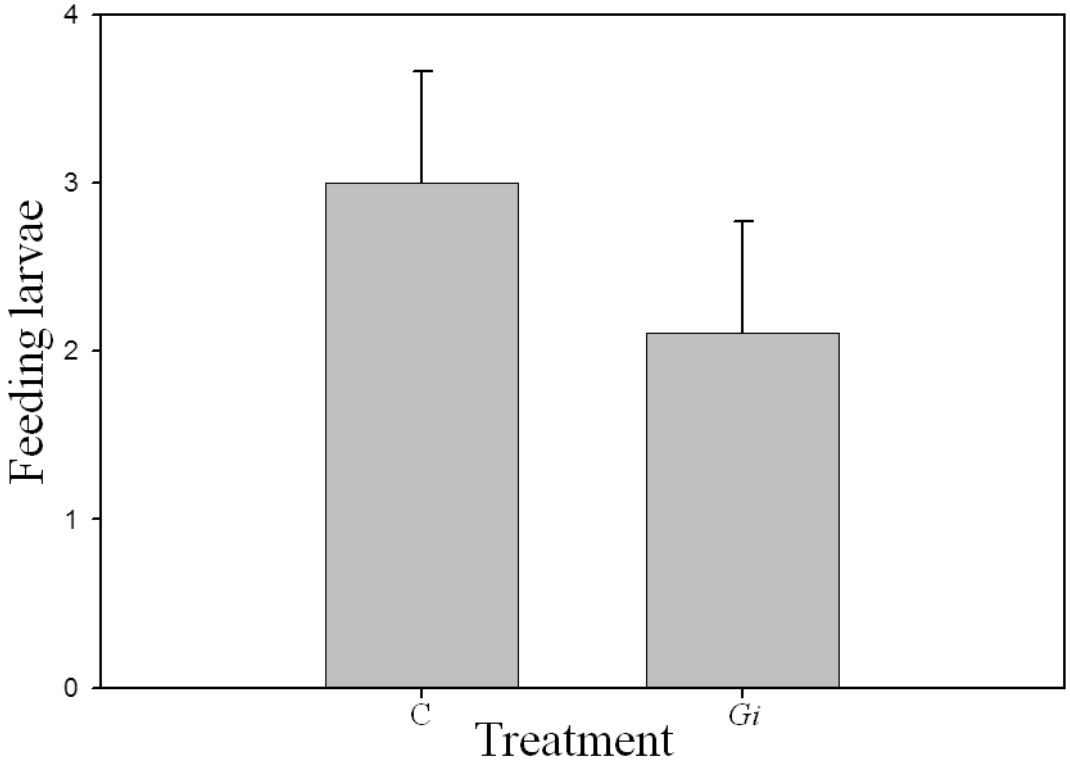

Fig. A.3. Effect of mycorrhizae on larvae of fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (choice test - pairs). Twenty-five (*S. frugiperda*) larvae were placed equidistant from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Numbers of larvae were counted during five days. Treatments: no-sorghum, no-mycorrhizae control (C); and sorghum colonized with *Glomus intraradices* (*Gi*). Bars without letters are not different according to an F-protected LSD (*P*= 0.3577).

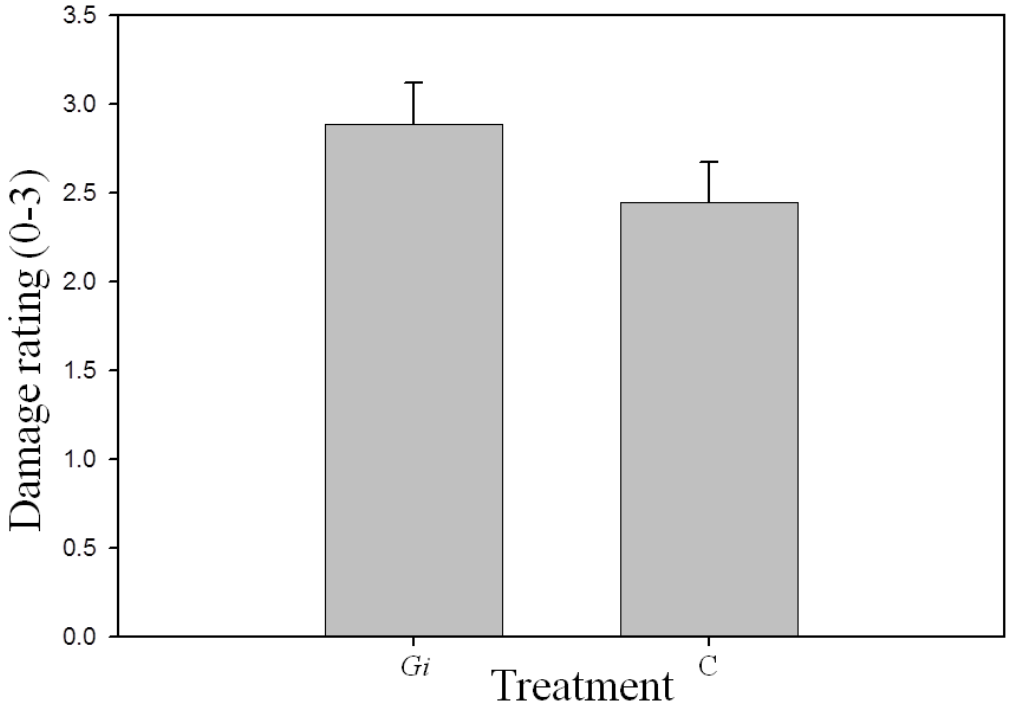

Fig. A.4. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) –rating scale. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Damage rating scale was 0 – 3 (Hardy et al., 1985). Image analysis damage estimate (%) was converted to the damage rating scale (0 – 3) developed by (Hardy et al., 1985). Treatments: no-sorghum, no-mycorrhizae control (C); and sorghum colonized with *Glomus intraradices* (*Gi*). Bars without letters are not different according to an F-protected LSD (*P*= 0.1755).

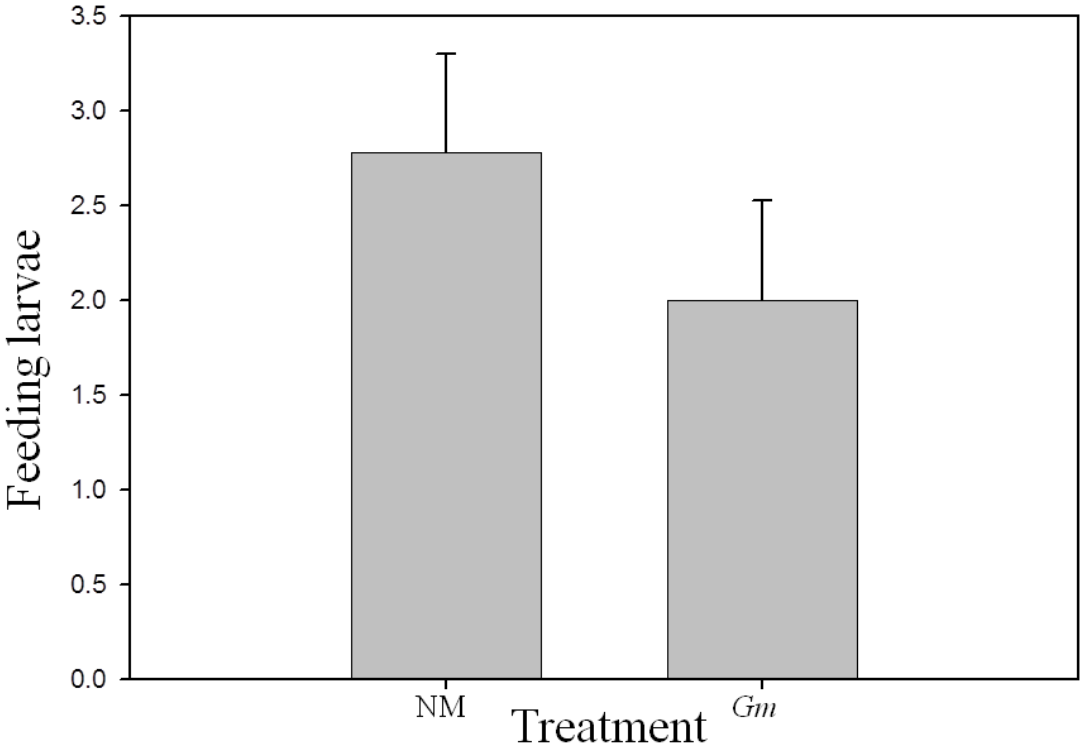

Fig. A.5. Effect of mycorrhizae on larvae of fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (choice test). Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Number of larvae were counted during five days. Treatments: non-mycorrhizal sorghum (NM); and sorghum colonized with *Gigaspora margarita* (*Gm*). Bars without letters are not different according to an F-protected LSD (*P*= 0.3118).

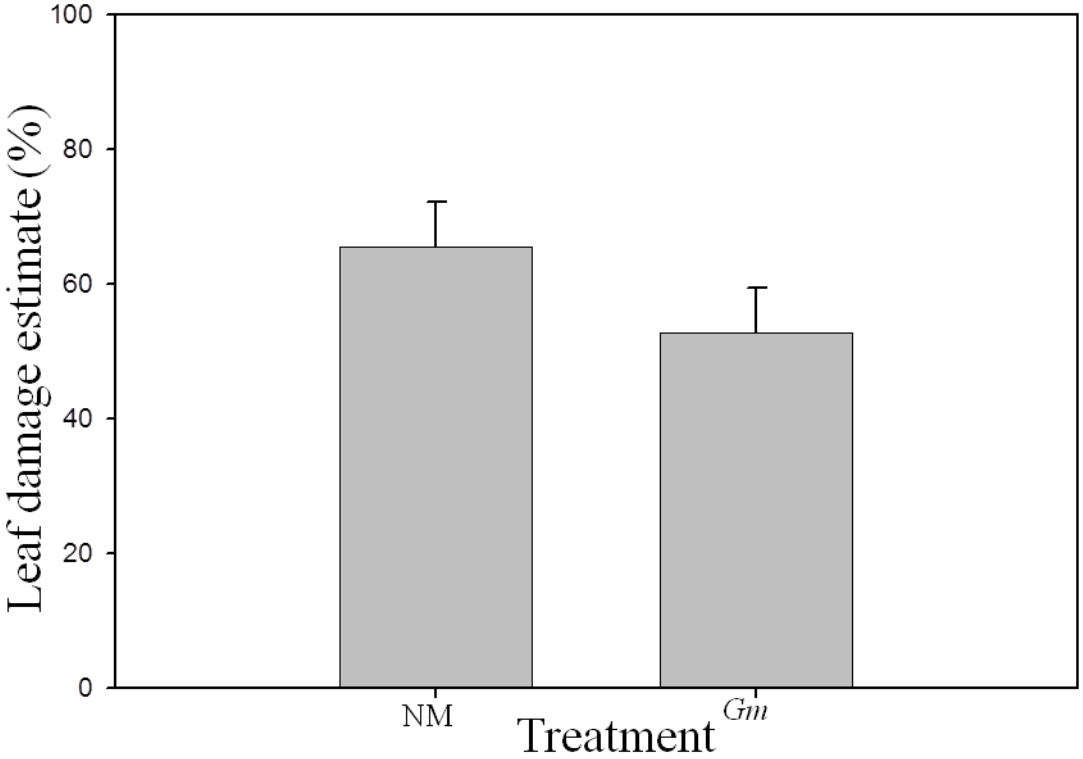

Fig. A.6. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) – subjective estimate of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Damage estimates are the mean of two raters' subjective scores. Treatments: non- mycorrhizal sorghum (NM); and sorghum colonized with *Gigaspora margarita* (*Gm*). Bars without letters are not different according to an F-protected LSD (*P*= 0.1877).

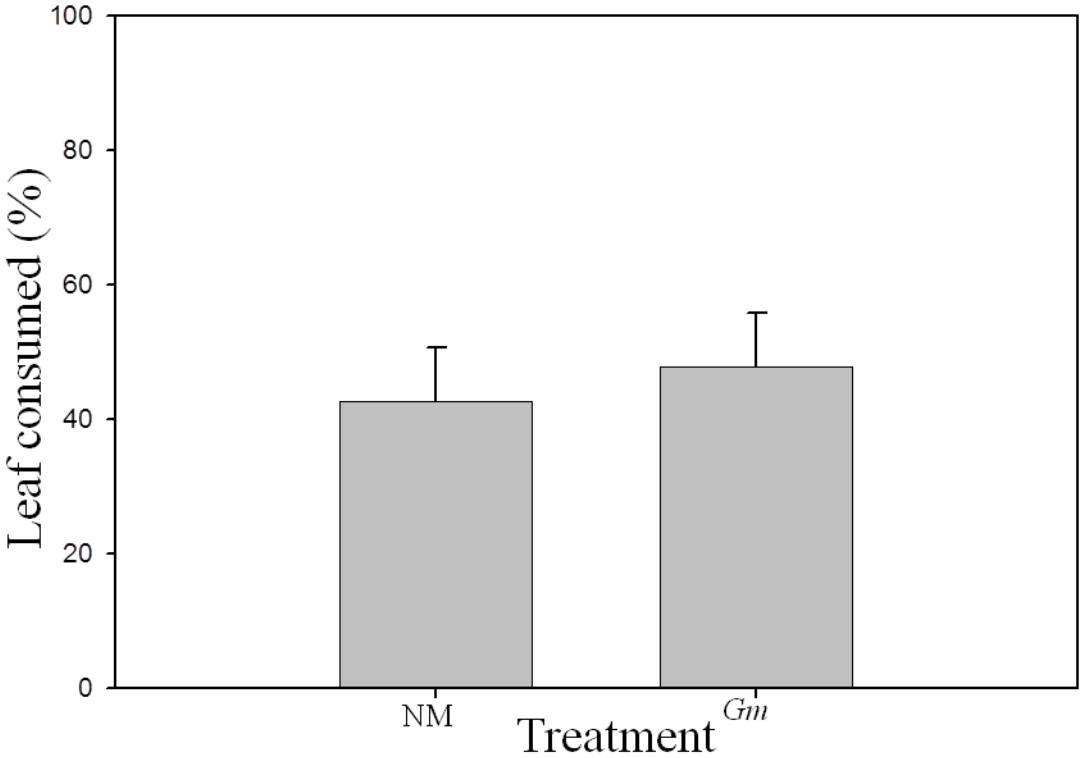

Fig. A.7. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) - image analysis of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Percentage consumption was determined using Assess 2.2 Image Analysis Software for Plant Disease Quantification. Treatments: non- mycorrhizal sorghum (NM); and sorghum colonized with *Gigaspora margarita* (*Gm*). Bars without letters are not different according to an F-protected LSD (*P*= 0.5631).

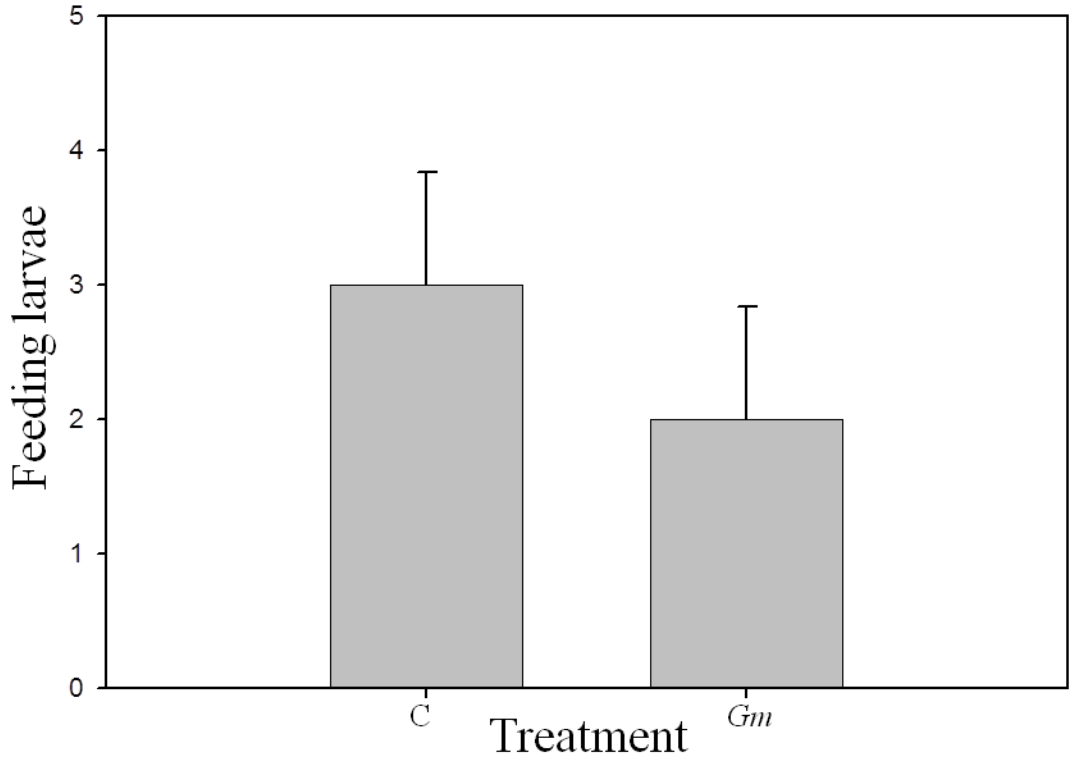

Fig. A.8. Effect of mycorrhizae on larvae of fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (choice test). Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Numbers of larvae were counted during five days. Treatments: no-mycorrhizae, no sorghum control (C); and sorghum colonized with *Gigaspora margarita* (*Gm*). Bars without letters are not different according to an F-protected LSD (*P*= 0.2462).

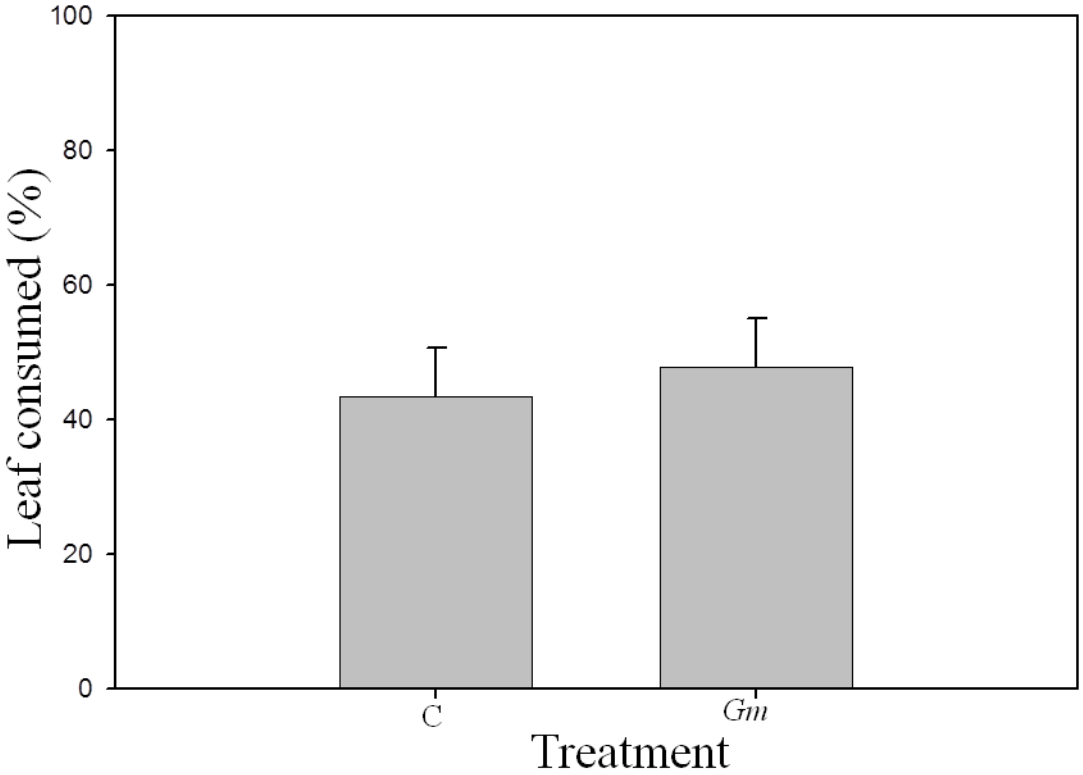

Fig. A.9. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test)- image analysis of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Percentage consumption was determined using Assess 2.2 Image Analysis Software for Plant Disease Quantification. Treatments: no- mycorrhizae, no sorghum control (C); and sorghum colonized with *Gigaspora margarita* (*Gm*). Bars without letters are not different according to an F-protected LSD (*P*= 0.5700).

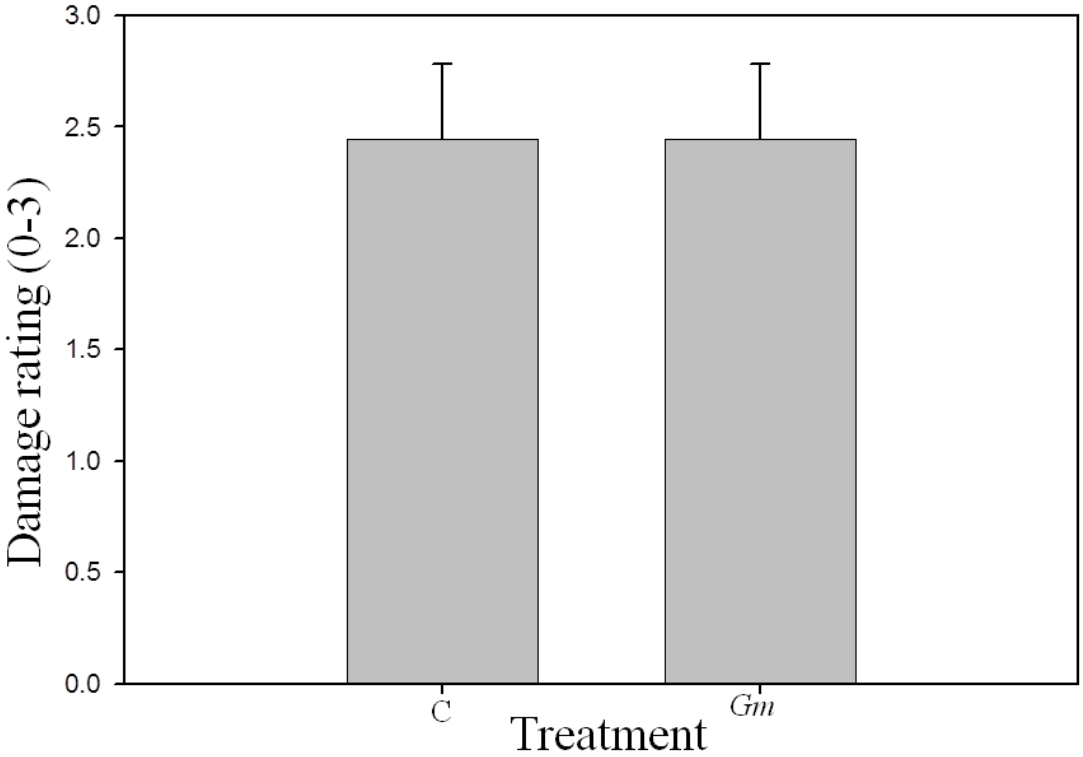

Fig. A.10. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) – rating scale. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Damage rating scale was 0 – 3 (Hardy et al., 1985). Image analysis damage estimate (%) was converted to the damage rating scale (0 – 3) developed by (Hardy et al., 1985). Treatments: no- mycorrhizae, no sorghum control (C); and sorghum colonized with *Gigaspora margarita* (*Gm*). Bars without letters are not different according to an F-protected LSD (*P*= 1.0000).

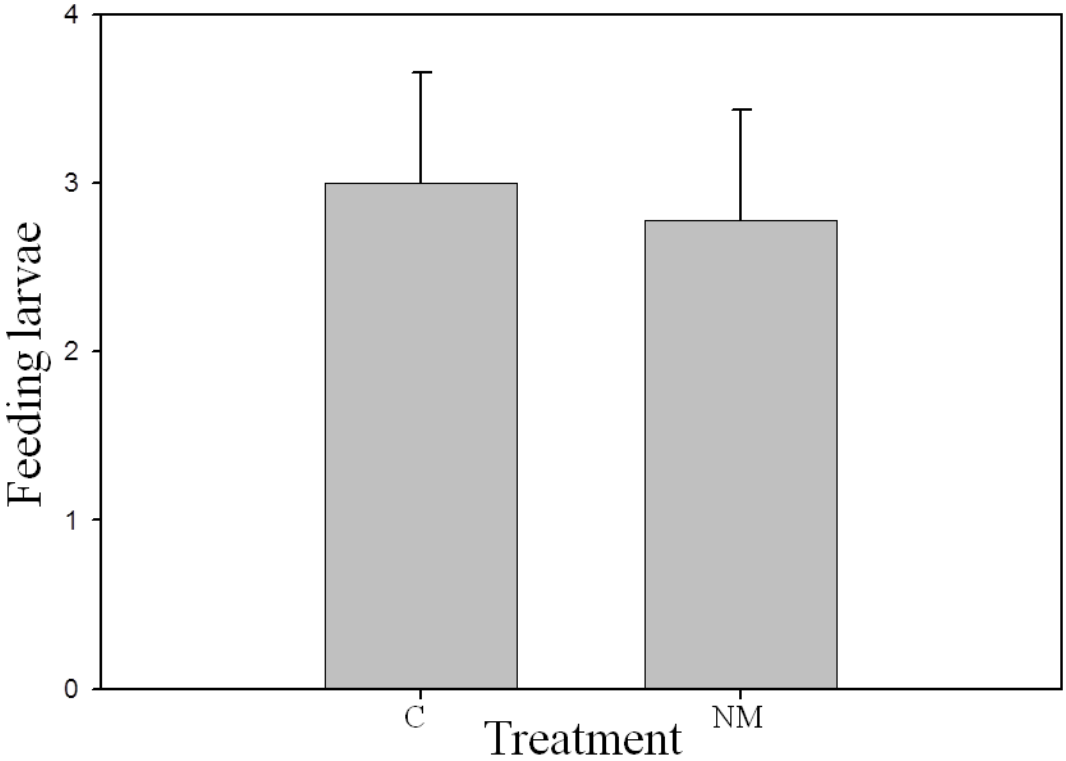

Fig. A.11. Effect of mycorrhizae on larvae of fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (choice test). Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Numbers of larvae were counted during five days. Treatments: no-mycorrhizae, no sorghum control (C); and non- mycorrhizal sorghum (NM). Bars without letters are not different according to an F-protected LSD (*P*= 0.8136).

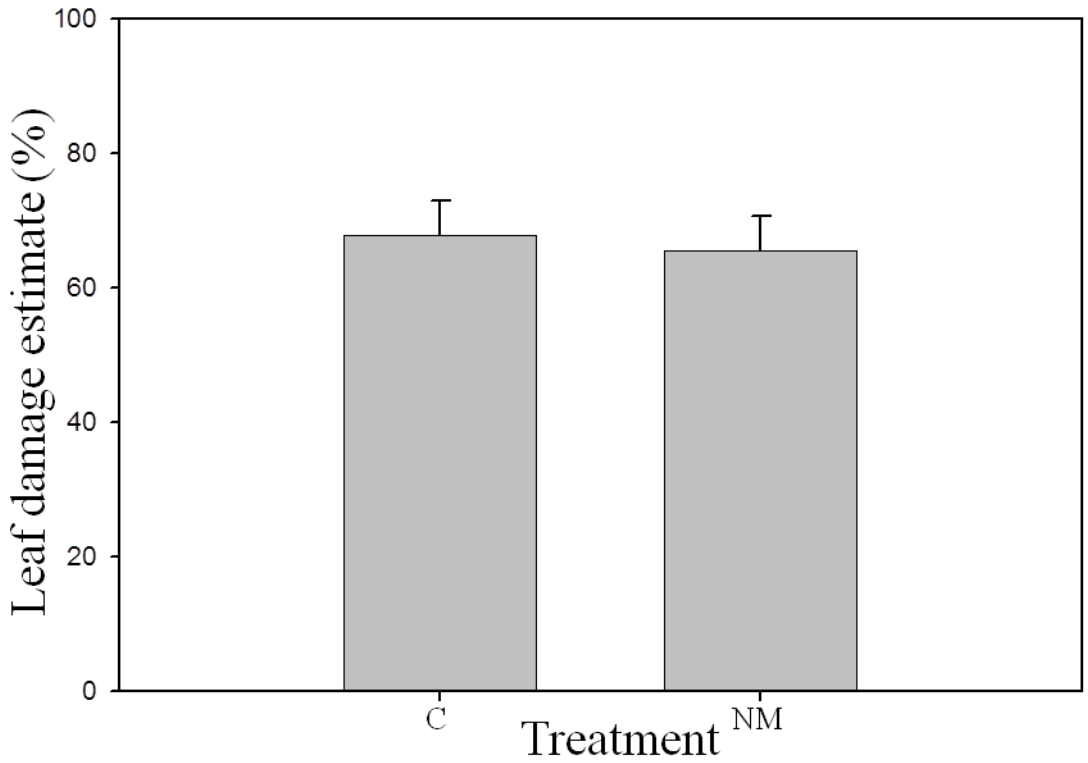

Fig. A.12. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) – subjective estimate of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Damage estimates are the mean of two raters' subjective scores. Treatments: no- mycorrhizae, no sorghum control (C); and non-mycorrhizal sorghum (NM). Bars without letters are not different according to an F-protected LSD (*P*= 0.7197).

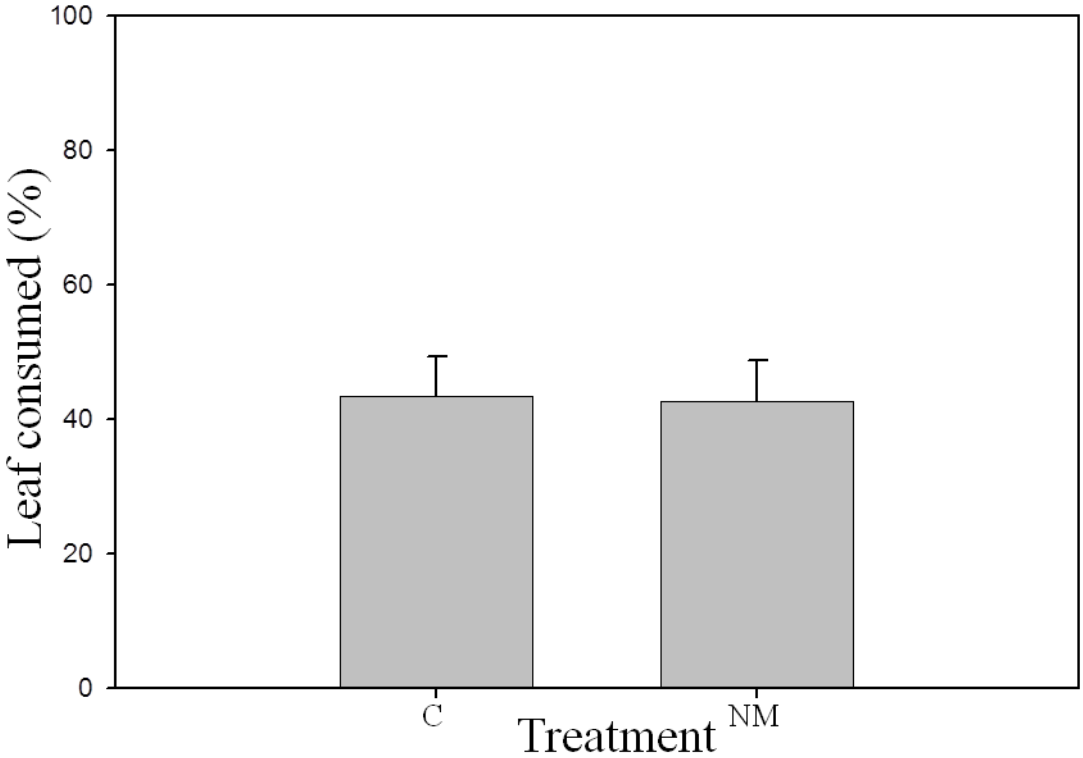

Fig. A.13. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test)- image analysis of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Percentage consumption was determined using Assess 2.2 Image Analysis Software for Plant Disease Quantification. Treatments: no- mycorrhizae, no sorghum control (C); and non- mycorrhizal sorghum (NM). Bars without letters are not different according to an F-protected LSD (*P*= 0.9346).

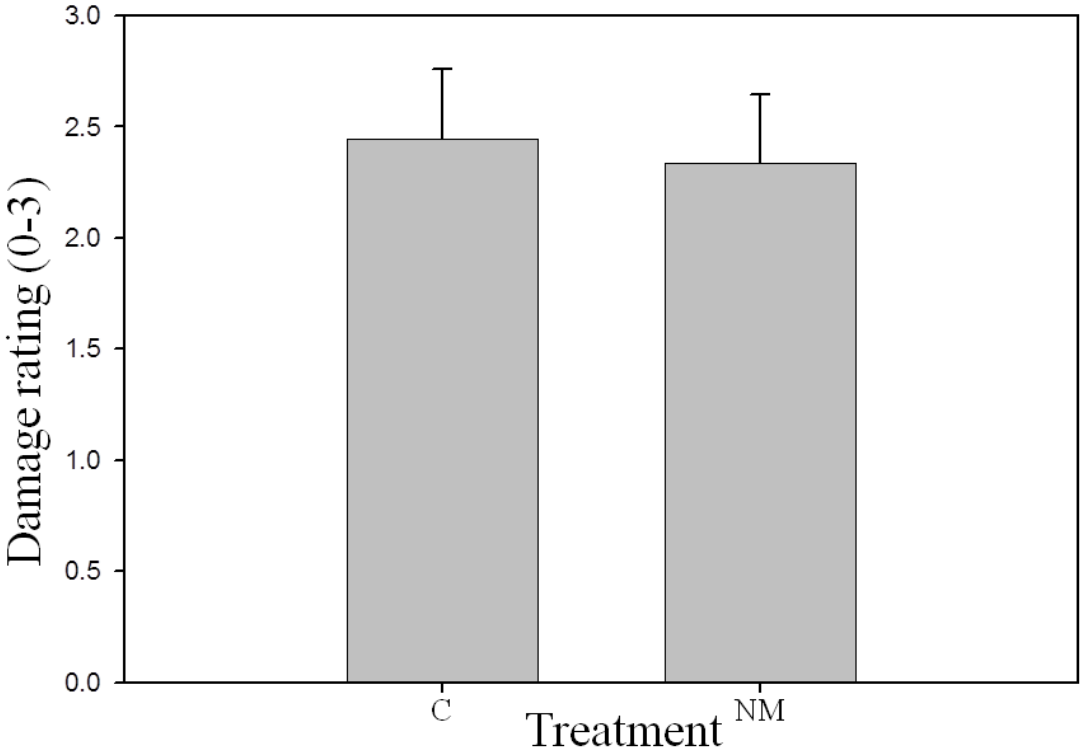

Fig. A.14. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) – rating scale. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Damage rating scale was 0 – 3 (Hardy et al., 1985). Image analysis damage estimate (%) was converted to the damage rating scale (0 – 3) developed by (Hardy et al., 1985). Treatments: no- mycorrhizae, no sorghum control (C); and non-mycorrhizal sorghum (NM). Bars without letters are not different according to an F-protected LSD (*P*= 0.8062).

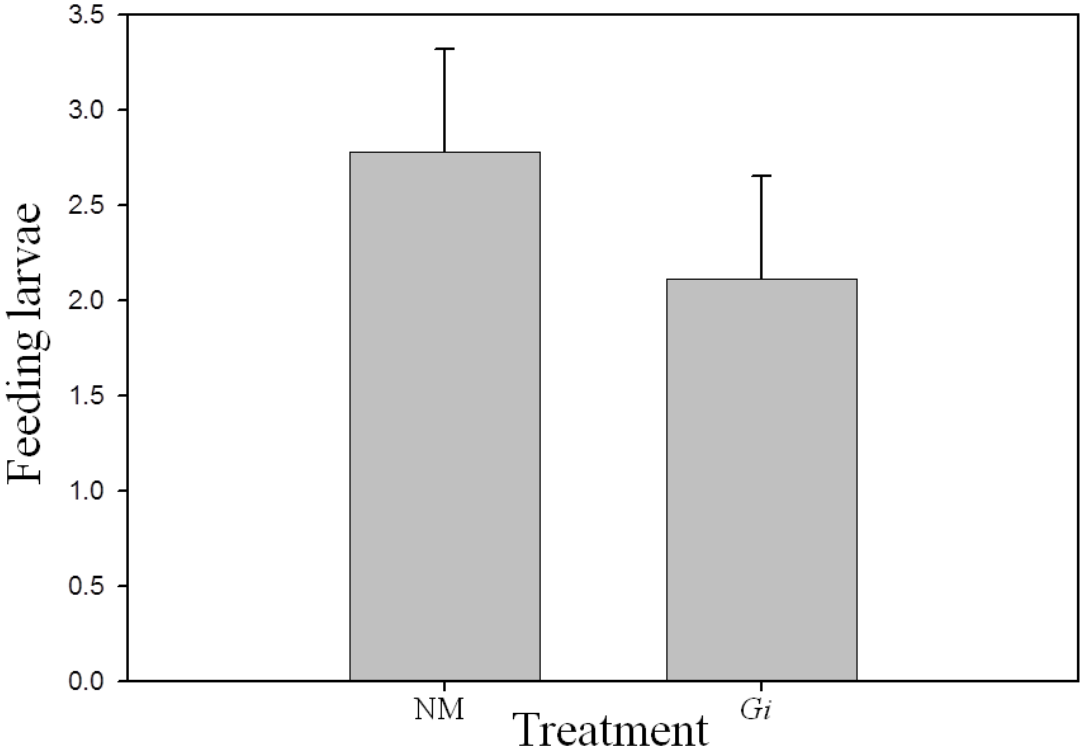

Fig. A.15. Effect of mycorrhizae on larvae of fall armyworm (FAW) (*Spodoptera frugiperda)* on wheat leaves (choice test). Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Numbers of larvae were counted during five days. Treatments: non-mycorrhizal sorghum (NM); and sorghum colonized with *Glomus intraradices* (*Gi*). Bars without letters are not different according to an F-protected LSD (*P*= 0.3862).

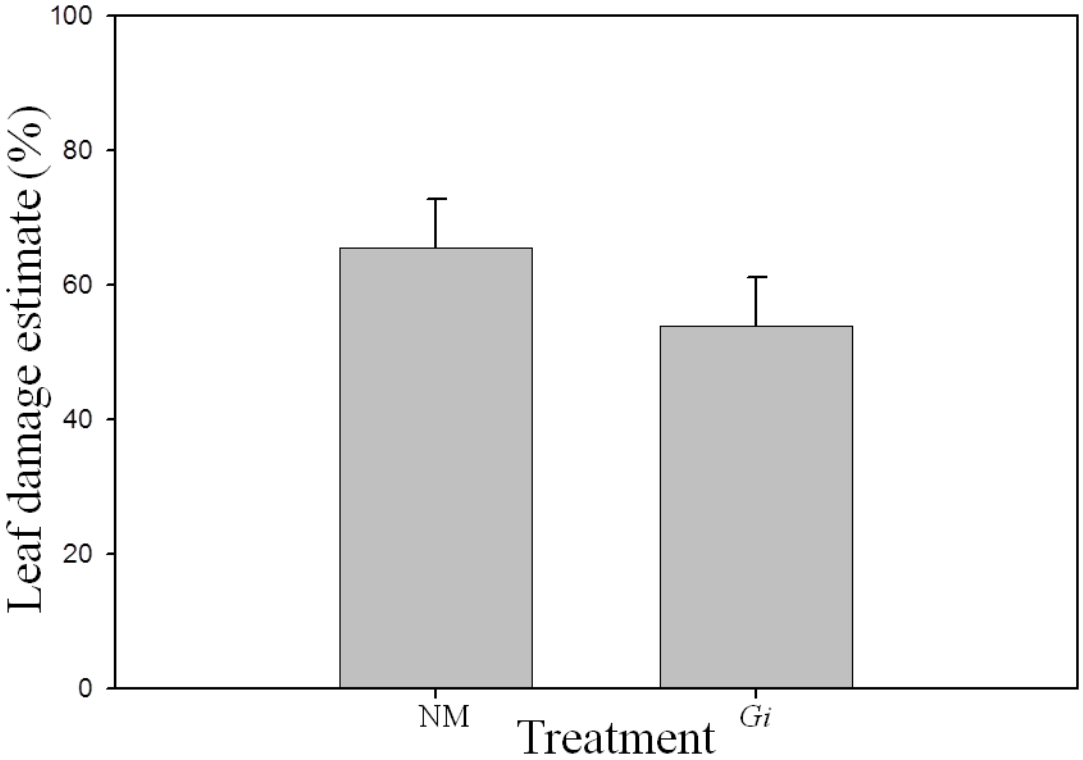

Fig. A.16. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) – subjective estimate of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Damage estimates are the mean of two raters' subjective scores. Treatments: non- mycorrhizal sorghum (NM); and sorghum colonized with *Glomus intraradices* (*Gi*). Bars without letters are not different according to an F-protected LSD (*P*= 0.7197).

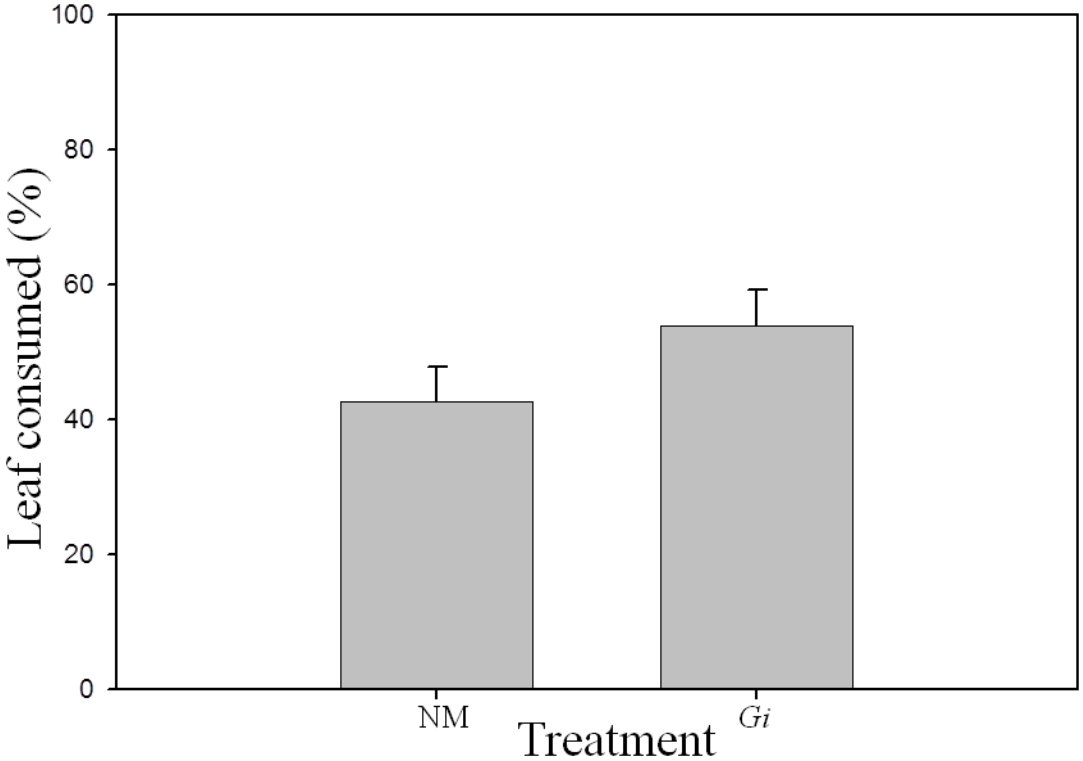

Fig. A.17. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) - image analysis of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings grown in substrate containing sorghum with or without mycorrhizae. Percentage consumption was determined using Assess 2.2 Image Analysis Software for Plant Disease Quantification. Treatments: non- mycorrhizal sorghum (NM); and sorghum colonized with *Glomus intraradices* (*Gi*). Bars without letters are not different according to an F-protected LSD (*P*= 0.1414).

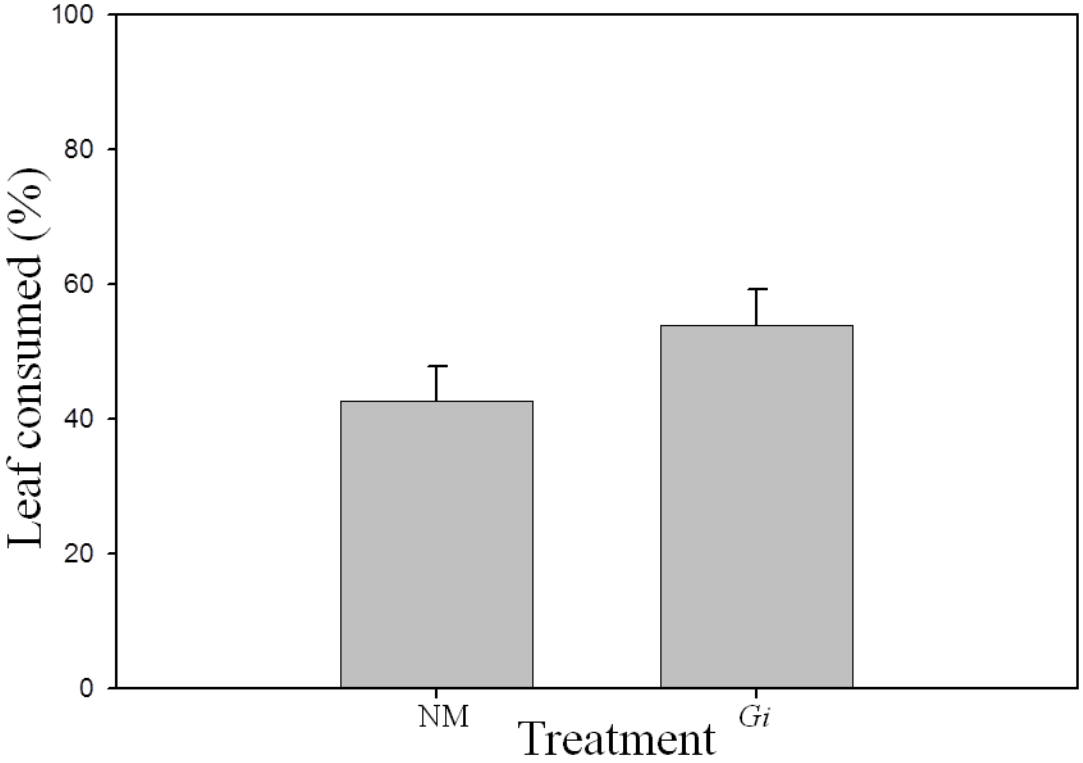

Fig. A.18. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (choice test) –rating scale. Twenty-five (*S. frugiperda*) larvae were placed from two leaf segments in an insect arena. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Damage rating scale was 0 – 3 (Hardy et al., 1985). Image analysis damage estimate (%) was converted to the damage rating scale (0 – 3) developed by (Hardy et al., 1985). Treatments: non- mycorrhizal sorghum (NM); and sorghum colonized with *Glomus intraradices* (*Gi*). Bars without letters are not different according to an F-protected LSD (*P*= 0.1362).

Appendix 4. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) feeding on wheat leaves (no-choice test).

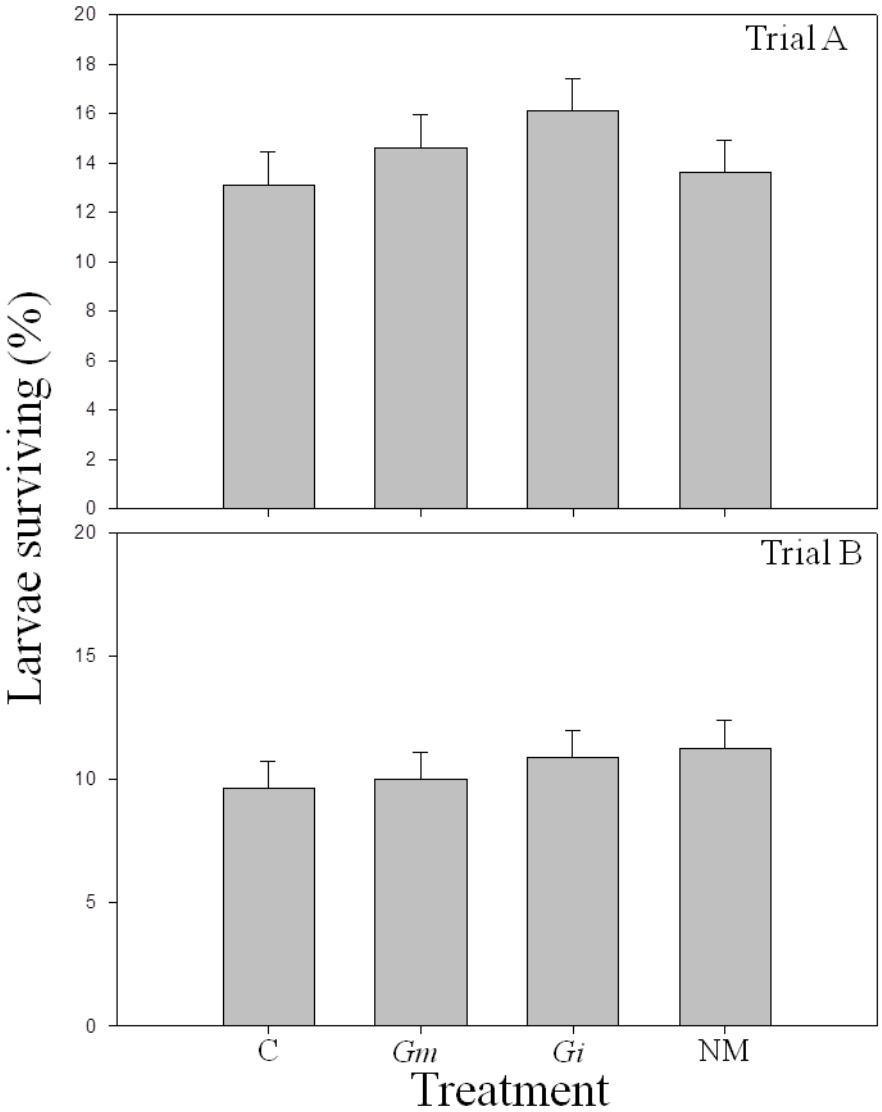

Fig. A.19. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) survivorship of wheat leaves (no-choice test). Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; each arena contained only one treatments. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Numbers of larvae were counted when visual estimate of leaf wheat displayed 50% reduction. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.361, Trial A; *P*= 0.722, Trial B).

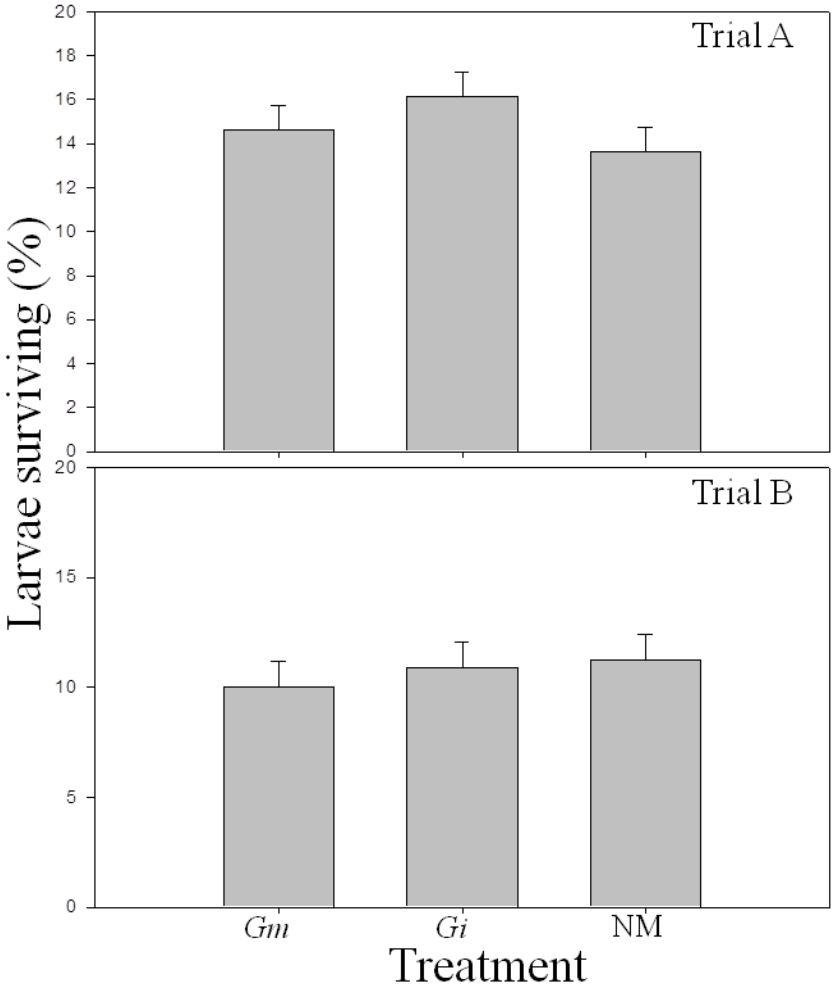

Fig. A.20. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) survivorship on wheat leaves (no-choice test) – without control. Twenty-five (*S. frugiperda*) larvae were placed from four leaf segments in an insect arena; each arena contained only one treatments. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Number of larvae were counted during five days. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.3195, Trial A; *P*= 0.722, Trial B).

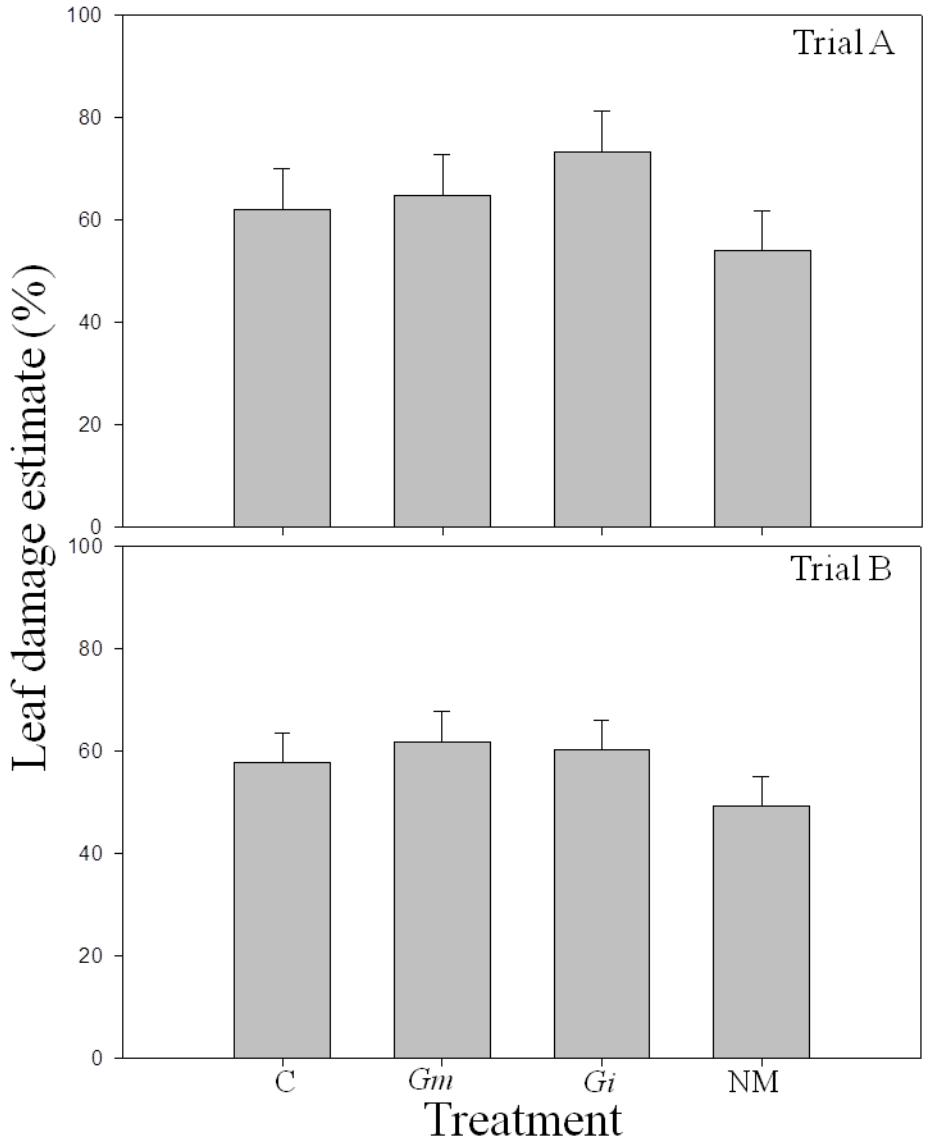

Fig. A.21. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (no-choice) – subjective estimate of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; each arena contained only one treatment. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Damage assessments are the mean of two raters' subjective scores. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.249, Trial A; *P*= 0.427, Trial B).

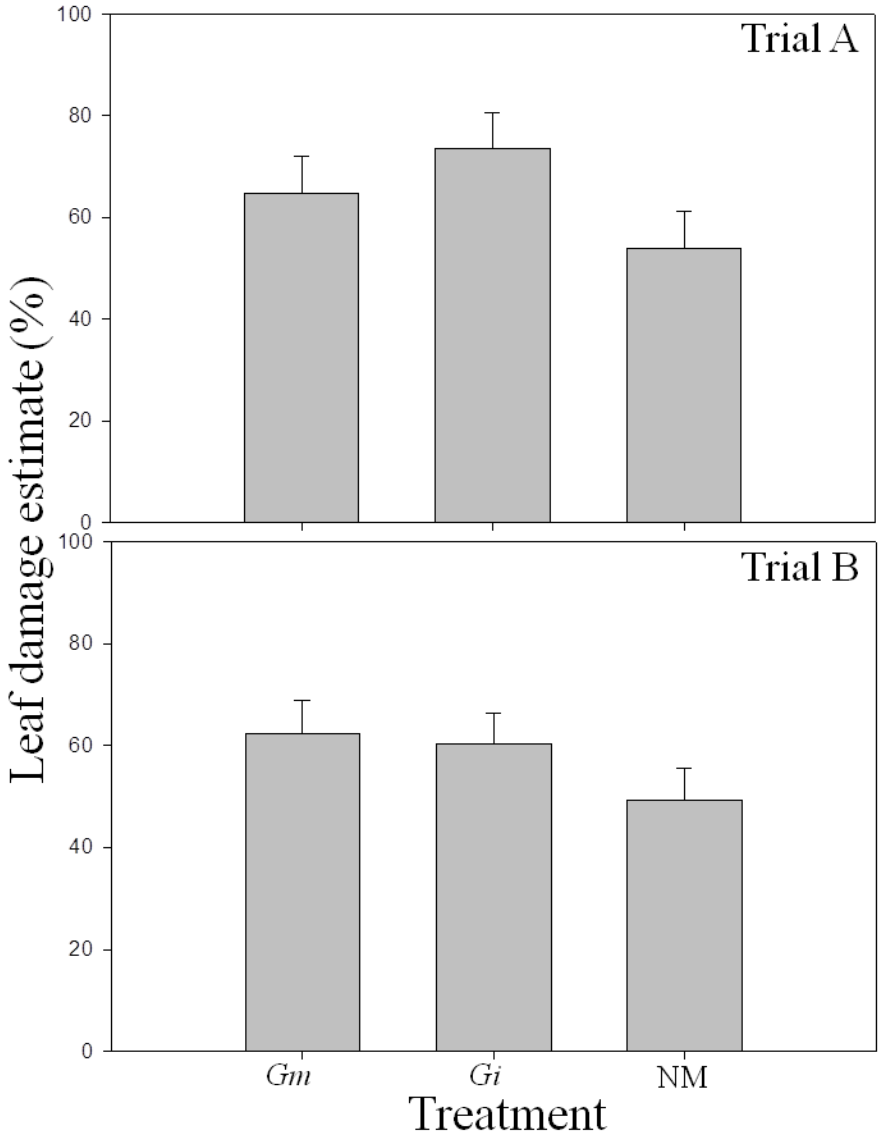

Fig. A.22. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (no-choice) – subjective estimate of leaf damage (without control). Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; each arena contained only one treatment. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Damage assessments are the mean of two raters' subjective scores. Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.160, Trial A; *P*= 0.243, Trial B).

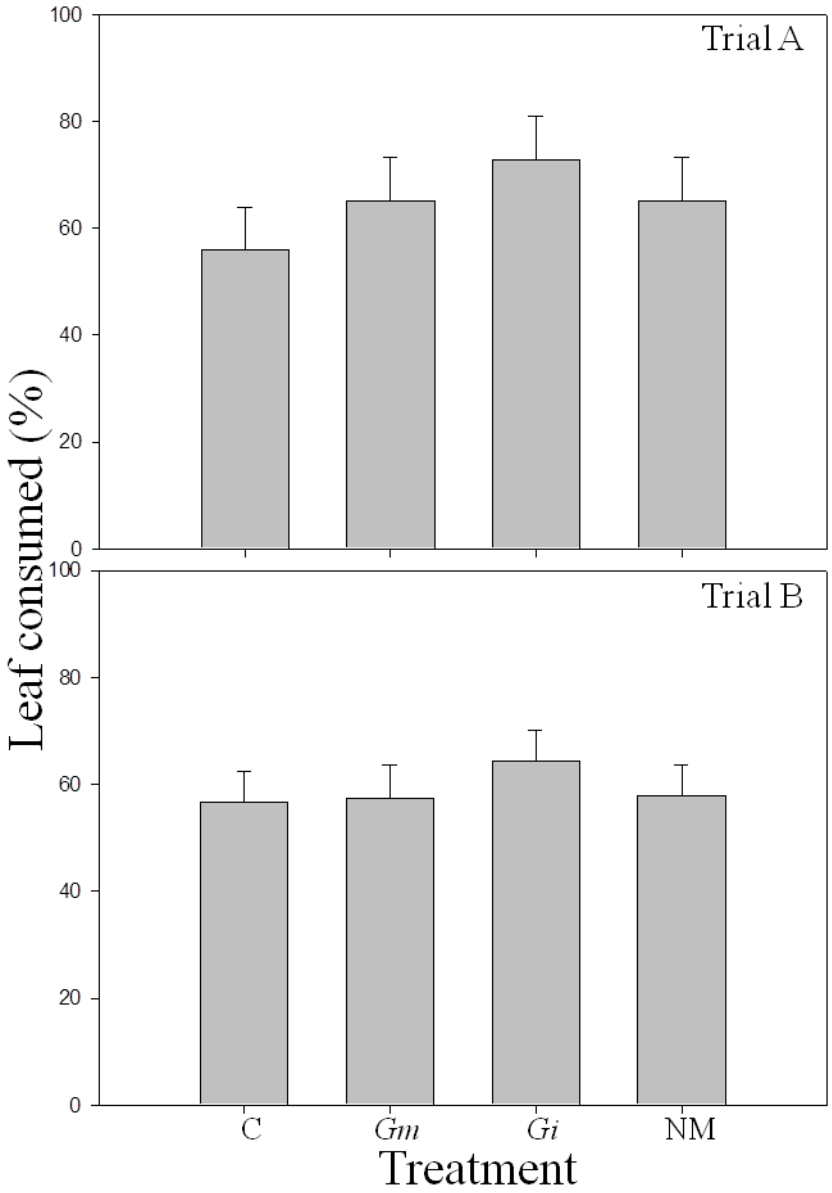

Fig. A.23. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (no-choice) – image analysis estimate of leaf damage. Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; each arena contained only one treatment. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Percentage consumption was determined using Assess 2.2 Image Analysis Software for Plant Disease Quantification. Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (P = 0.304, Trial A; P = 0.494, Trial B).

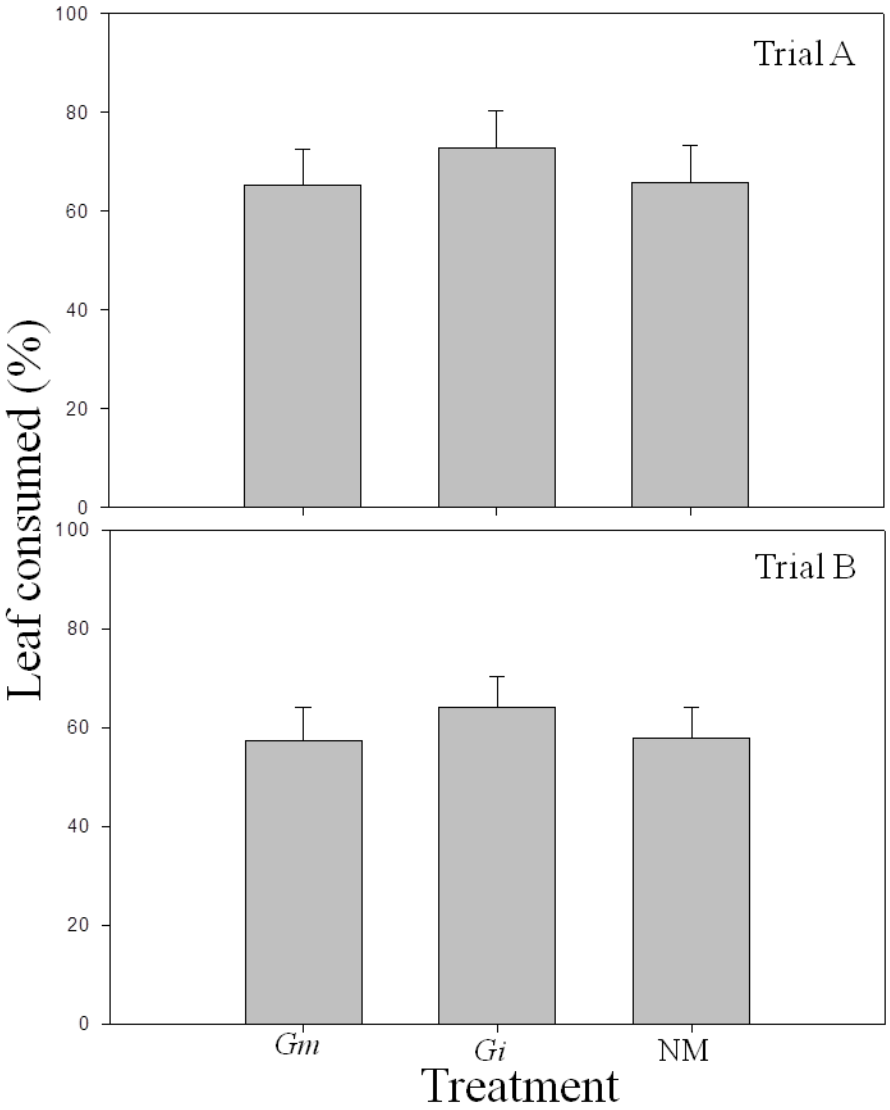

Fig. A.24. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (no-choice) - image analysis estimate of leaf damage (without control). Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; each arena contained only one treatment. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Percentage consumption was determined using Assess 2.2 Image Analysis Software for Plant Disease Quantification. Treatments are: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.726, Trial A; *P*= 0.701, Trial B).

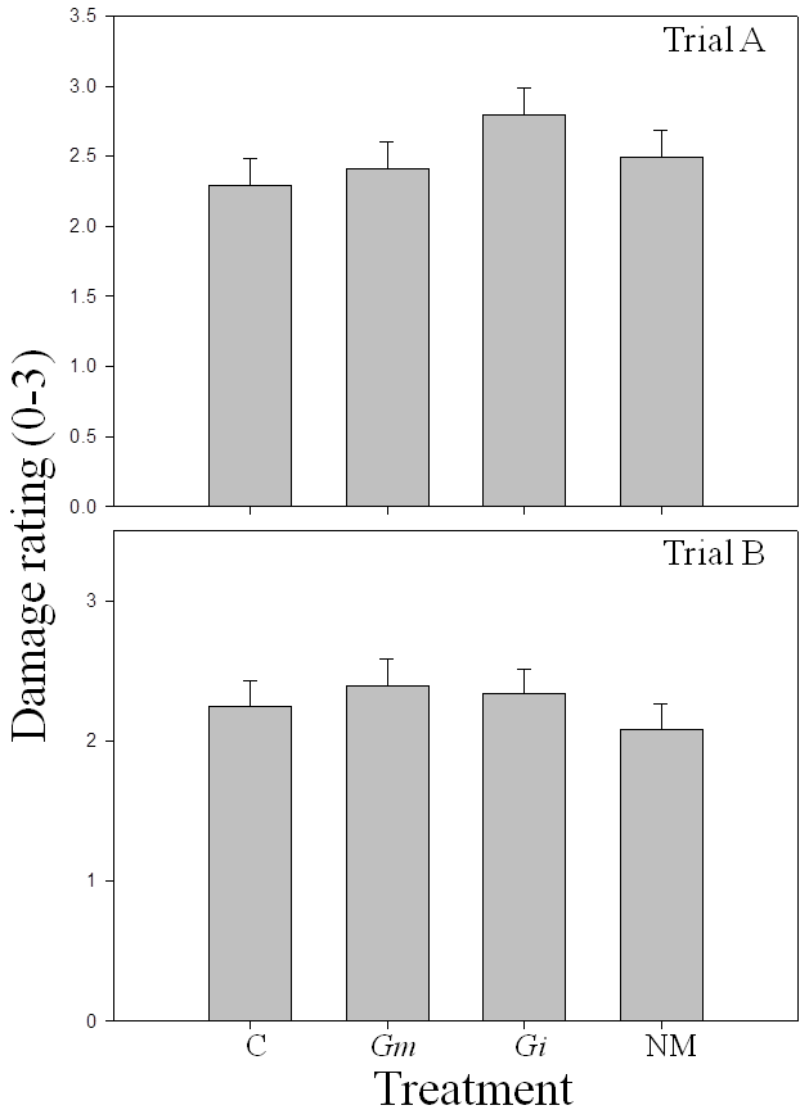

Fig. A.25. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (no-choice test) – subjective rating scale. Twenty-five (*S. frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; each arena contained only one treatment. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Image analysis damage estimate (%) was converted to the damage rating scale (0 – 3) developed by (Hardy et al., 1985). Treatments: no-sorghum, no-mycorrhizae control (C); sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.260, Trial A; *P*= 0.644, Trial B).

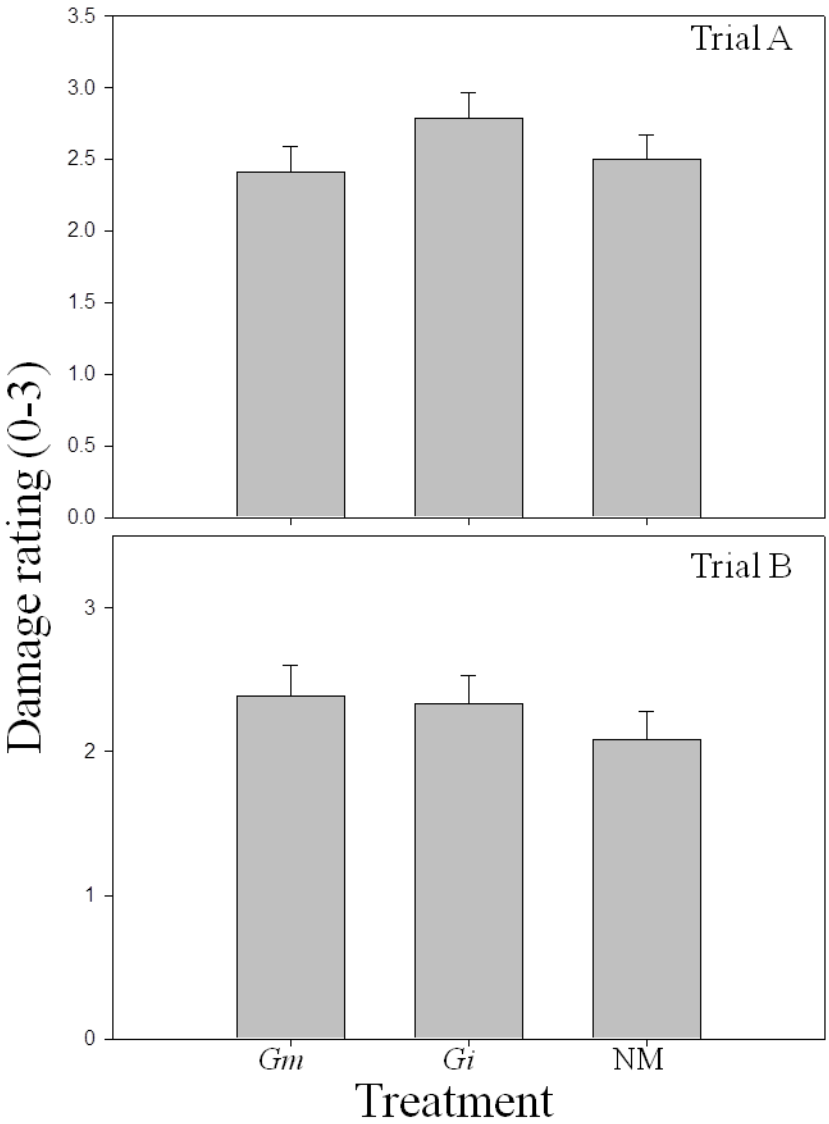

Fig. A.26. Effect of mycorrhizae on fall armyworm (FAW) (*Spodoptera frugiperda*) on wheat leaves (no-choice test) – subjective rating scale (without control). Twenty-five (*S.frugiperda*) larvae were placed equidistant from four leaf segments in an insect arena; each arena contained only one treatment. Excised leaves were obtained from 4-week-old wheat seedlings that were grown in substrate containing sorghum with or without mycorrhizae. Image analysis damage estimate (%) was converted to the damage rating scale (0 – 3) developed by (Hardy et al., 1985). Treatments: sorghum colonized with *Gigaspora margarita* (*Gm*); sorghum colonized with *Glomus intraradices* (*Gi*); and non-mycorrhizal sorghum (NM). Within each trial, bars without letters are not different according to an F-protected LSD (*P*= 0.304, Trial A; *P*= 0.494, Trial B).