BRASSICA BIOFUMIGATION FOR MANAGEMENT OF RICE SHEATH BLIGHT CAUSED BY *RHIZOCTONIA SOLANI* AG 1-1A.

A Dissertation

by

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DOCTOR OF PHILOSOPHY

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Major Subject: Plant Pathology

ABSTRACT

Sheath blight caused by *Rhizoctonia solani* is one of the most important rice disease that can cause significant losses in grain yield and quality in the United States. Current management options for sheath blight consist of fungicides, tolerant cultivars and cultural practices. These options are not always very effective. *Brassica* plants have been used for soil fumigation to manage a variety of soilborne pathogens. Eleven plant species comprising of six *Brassica juncea* cultivars (Brand 199, Ruby Streak, Florida Broadleaf, Green wave, Red giant and Sheali Hong), two *Brassica rapa* cultivars (Southern Green and Napa), *Brassica oleracea*, *Eruca sativa* and *Crotalaria juncea* were evaluated for their efficacy on *R. solani* mycelia inhibition with or without soils collected from Texas, Arkansas or Mississippi rice fields. Evaluation of allyl isothiocynates (AITC) emission during the process of biofumigation was achieved by mixing *B. juncea* plant tissue at a rate of 0.25 or 0.5% (wt/wt) with either natural soil or pasteurized soil. The impacts of *B. juncea* soil amendment on the viability, aggressiveness, and sclerotia formation of *R.* s*olani* AG1-1A was evaluated. *B. juncea* cover crop was integrated with host resistance and fungicide application to evaluate impact on rice sheath blight severity and rice yield.

Four *B. juncea* cultivars (Brand 199, Ruby Streak, Florida Broadleaf, Green wave) consistently provided the greatest inhibition in all the soil types tested. Mycelial inhibition increased with an increase of *B. juncea* application rate. Plant tissue mixed with natural soils resulted in significantly higher AITC emission than in pasteurized soils.

B. juncea amendment reduced numbers of sclerotia formed from the mycelium exposed to soils amended with *B. juncea*. Viability and aggressiveness of sclerotia or mycelium was reduced in soils amended with 3.2 % (wt/wt) of *B. juncea*. *B. juncea* cover crop significantly lowered sheath blight severity in all three years and led to a significantly higher grain yield in 2013 as compared to the fallow control. *B. juncea* cover cropping can be added to the rice disease management practices to enhance the efficacy of the current integrated pest management program for sustainable control of sheath blight in rice.

DEDICATION

To my mom and dad who inspired me to persue a higher education and yet they could not wait to see me get it done. I promise to pass on the inspiration to your grandchildren!!

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Rice (*Oryza sativa* L.) is one of the most important food crops in fighting against global hunger. Over half of the world's population depend on rice as their staple diet. The United States rice industry produced more than 958, 000 tons from more than 997, 000 ha in 2013 (USDA, 2014).

One of the major diseases limiting rice production in the southern United States is sheath blight, caused by *Rhizoctonia solani* Kühn AG1-1A [teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk] (Groth and Lee 2003). Sheath blight can cause as much as 50% yield loss and significant loss in grain quality (Groth 2005). The pathogen overwinters in infected plant tissue as sclerotia and mycelium, which serve as the primary inoculum and may survive in the soil for up to two years (Groth 2005). It has a wide host range comprised of both monocots and dicots (Larkin and Griffin 2007) including soybean. Many rice farmers in the United States rotate rice and soybean, both of which serve as hosts for *R. solani*. There has been an increase in sheath blight incidence and severity, and this has been attributed to modern rice production practices such as large-scale monoculture, use of semi-dwarf cultivars, high nitrogen fertilization, and high plant populations (Groth and Lee 2003). There are no commercial rice cultivars highly resistant to sheath blight. Therefore, sheath blight management practices have been focused on the use of tolerant varieties, optimized nitrogen fertility, proper seeding rates and inoculum-reducing practices such as rotation, cultivation and sanitation (Groth and Lee 2003;Groth

2005; Kumar et al. 2009). Given the application of these cultural disease management practices, rice farmers in the United States still heavily rely on synthetic fungicides. Intensified and large-scale use of fungicides can result in negative impacts on the environment (Bunemann et al. 2006; Gullino and Kuijpers 1994). The interest in using environment-friendly management practices has increased in the past years.

Biologically based treatments such as use of organic residue have been promoted as alternatives to the use of broad spectrum biocides for the management of soilborne pathogens (Lazzeri et al. 2003; Pascual et al. 2004). Soil amendments with organic residue can modify the microbial community composition and can enhance the competition and/or antagonism among soil borne microbes, leading to a decrease in soil borne pathogen activity (Hoitink and Boehm, 1999; Steinberg et al., 2004). Changes in the microflora composition and dynamics resulting from resource competition has been detected following soil amendments with organic residue (Vaughn, 1993; Smolinska et al., 1997; Shetty et al., 2000).

Rhizoctonia species in apple and potato cropping systems have been reported to be effectively managed using biologically based soil amendments (Mazzola et al. 2007; Larkin and Griffin 2007). The production and yield of various bioactive chemistries including glucosinolate hydrolysis products, has encouraged studies exploring pest control using *Brassicaceae* plant residue (Mazzola et al. 2007). *Brassicaceae* species have been incorporated into the soil as a source of biofumigation and showed positive effects on combating soilborne diseases in various field crops (Angus et al. 1994; Matthiessen and Kirkegaard 2006; Kirkergaard et al. 2000; Yulianti et al. 2006; Brown

and Morra 1996; Larkin and Griffin 2007; Matthiessen and Kirkegaard 2006; Smolinska and Horbowicz 1999).

The pesticidal effect of the *Brassica* plant is based on the production of toxic and volatile isothiocyanates upon hydrolysis by myrosinases. Isothiocyanates have biocidal activity toward numerous plant pests (Brown and Morra 1996), parasitic nematodes (Subbarao et al. 1999; Henderson et al. 2009; Izzo and Mazzola 2006), and plant pathogenic fungi and oomycetes (Manici et al. 1997; Mazzola et al. 2007). Efficacy of *Brassica* plant tissue has been reported against verticillium wilt (*Verticillium dahlia*) on cauliflower (*Brassica oleracea* var. *botrytis*) (Subbarao et al. 1999), *Sclerotinia sclerotiorum* (Smolinska and Horbowicz 1999), *Verticillium dahliae* (Oliver et al. 1999), take-all (*Gaeumannomyces graminis*) on wheat (*Triticum aestivum*) (Kirkegaard et al. 2000), and Sclerotinia drop (*Sclerotinia minor*) on lettuce (*Lactuca sativa*) (Hao et al. 2003).

Ample evidence exists to support the model that brassica biofumigation has a chemistry derived pest suppression (Matthiessen and Kierkegaard 2006). However, several recent studies reveal that additional mechanisms may operate in concert with or independent of these chemistries (Mazola et al. 2012). *Brassicaceae* amendments may require activity of the resident soil biology and not only the generation of glucosinolate hydrolysis products (Mazzola et al. 2007; Hoagland et al. 2008; Friberg et al. 2009; Motisi et al. 2009). The efficacy of soil amendments with *Brassica* residue on soilborne pathogens has been attributed to altered soil biology (Cohen et al. 2005; Mazzola et al. 2007; Hoagland et al. 2008; Frieberg et al. 2009). The mechanism of action in disease

suppression varies in pathogen specific manner and includes both chemical and biological modes (Mazzola et al. 2007).

Incorporation of plant materials into soil is one of the major routes employed in the use of *Brassica* species as a source of biological soil fumigation (Angus et al. 1994; Kirkegaard et al. 1996; Charron and Sams 1999; Lazzeri et al. 2003). Techniques such as use of *Brassica* seed meals and macerated *Brassica* plant tissue have been developed to achieve soil fumigation. Benefits of Brassica biofumigation are related to increased soil organic matter and improved soil structure as well as biocidal effects of soilborne pathogens (Larkin and Griffin 2007). However, different species or cultivars of *Brassica* plants have different efficacy in biofumigation due to varying concentrations and chemical types of the glucosinolates in plant tissue (Kirkergaard et al. 1996; Kirkergaard and Sarwar 1996). Kirkegaard et al. (1996) concluded that the degree of fungal suppression by *Brassica* plants was related to the concentration and type of biocidal volatile gases released from plant tissue. Previous *in vitro* bioassays has shown varying effects of *Brassica* species on suppressing growth of several fungi (Smith and Kierkegaard 2002; Sarwar et al. 1998; Charron and Sams 1999; Lazzeri and Manici 2001; Yulianti et al. 2006). However, the *in vitro* bioassays failed to predict efficacy of *Brassica* plants under field conditions because their methodologies of screening excluded the role of soil properties and soil microbes in the process of biofumigation. Gimsing et al. (2006) reported microbial myrosinase production in soil influences the *Brassica* biofumigation process, emphasizing the importance of soil properties and microbial activities.

A number of studies have demonstrated the potential use of brassicaceous biofumigants to control soilborne plant pathogens and diseases such as *Rhizoctonia* spp. and take-all diseases of wheat (Murray and Brenna 1988; Charron and Sams 1999), nematodes and weeds (Matthiessen and Kirkegaard 2006). However, inconsistencies in the effectiveness of brassicaceous biofumigation have been also reported in some crop systems (Njoroge et al. 2008; Motisi et al. 2009; Motisi et al. 2010). This is, in part, due to differences in sensitivity of propagules or survival forms of soilborne pathogens to biofumigants. The tolerance of the resting structures of pathogens to the allyl isothiocyanates (AITCs) released from *Brassica* plant tissue is related to the failure of biofumigation in disease suppression (Yulianti et al. 2008). Previous studies have focused on evaluating the *in vitro* effects of AITCs released from macerated *B. juncea* plant tissue on the growth of mycelium of plant pathogenic fungi including *R. solani* (Mayton, et al. 1996; Yulianti 2008; Zhou et al. 2011). There is a dearth of information on the impacts of *B. juncea* soil amendment on the mycelium and sclerotia of *R. solani* AG1-1A, which is crucial to the development of an effective brassicaceous biofumigation approach for management of sheath blight in rice. There are no published records of the management of rice sheath blight using biofumigation to date, even though biofumigation has been reported to be effective in other *R. solani* groups and cropping systems. The objectives of this study are to identify a *Brassica* cover crop suitable biofumigation of *R. solani* AG1-1A, understand how its application rate influences inoculum viability and aggressiveness and integration of biofumigation, host resistant and fungicide application to manage rice sheath blight.

SPECIFIC OBJECTIVES

- Identify a biofumigant *Brassica* crop with the most growth inhibition on *R. solani* AG1-1A *in-vitro*.
- Investigate whether the release of allyl isothiocyanate (AITC) from *Brassica* tissue amended soils depends on soil properties.
- Investigate the impact of amending soil with *B. juncea* at different application rates on mycelia growth, sclerotia viability and aggressiveness on rice plant tissue.
- Evaluate the impact of *B. juncea* amendment, host resistance and fungicide application on *R. solani* management and yield.

HYPOTHESIS

- High allyl glucosinolate containing *Brassica* crop has the most growth inhibition effect on *R. solani* AG1-1A in-vitro.
- Allyl isothiocyanate release from brassica amended soil is affected more with soil properties.
- *R. solani* AG1-IA mycelia growth inhibition and sclerotia viability are *B. juncea* application rate dependent under controlled environment.
- Integrating *B. juncea* biofumigation, with host resistance and fungicide application results in reduced rice sheath disease severity.

CHAPTER II

SCREENING BRASSICACEOUS PLANTS AS BIOFUMIGANTS FOR MANAGEMENT OF *RHIZOCTONIA SOLANI* AGI-IA

OVERVIEW

Brassicaceous plants have been used as biofumigants for management of *Rhizoctonia solani* and other soilborne pathogens in a variety of agricultural crops. Biofumigation to manage R. *solani* AG 1-1A, the causal agent of sheath blight in rice needs more validation. In this study, biofumigation activities of nine brassicaceous plants and two other related species were evaluated *in vitro* with soils from Texas, Arkansas or Mississippi. The results showed that all plants evaluated significantly suppressed the mycelium growth of *R. solani* AG 1-IA. Four mustard (*Brassica juncea*) cultivars (Brand 199, Ruby Streak, Florida Broadleaf, and Green Wave) consistently provided the greatest (> 90%) mycelial inhibition in all three soils, while sunn hemp (*Crotalaria juncea*) and Chinese cabbage (*B. rapa*) had the least suppressive effect. The remaining plant species [*B. juncea* cvs. Red Giant and Sheali Hong, turnip (*B. rapa*), kale (*B. oleraceae*) and arugula (*Eruca sativa*)] showed intermediate efficacy (40 to 20%) or were inconsistent in the soils evaluated. In further evaluations, effects of soil pasteurization and plant tissue amendment rates were examined. Inhibition of mycelial growth became greater with the increase of plant amendment rate up to 3.2% (wt/wt) in the soil. Soil pasteurization almost completely suppressed the release of allyl isothiocyanate (AITC). The natural (non pasteurized) soil amended with 5% (wt/wt) of the plant material released 96% more

AITC than the soil amended with 2.5% (wt/wt) of the plant material. The highest level of AITC release was observed at 12 and 24 h after soil amendment with 2.5 and 5% (wt/wt) of the plant material respectively. Antifungal effects of *B. juncea* are attributed to dose-dependent production of volatile AITC and can be used for managing rice sheath blight caused by *R. solani* AG 1-IA

INTRODUCTION

Modern intensive crop farming systems with short crop rotations tend to result in an increase in inoculum densities of soilborne pathogens (Hassan and Yousif 2010). In rice (*Oryza sativa*) production, accumulation of soilborne inoculum of *Rhizoctonia solani* AG1-IA, the causal agent of sheath blight, can lead to severe disease outbreaks and consequent yield losses. Sheath blight is one of the major rice disease in the U.S. causing varying degrees of grain yield and quality losses every year. Conventional management of sheath blight is dependent on cultural practices and synthetic fungicides. However, heavy dependence on the use of synthetic fungicides causes adverse impacts on the human health and the environment. The interest of using environment-friendly management practices for sustainable rice production has increased in recent years.

Biological treatments such as use of plant organic residue can be an alternative to synthetic pesticides for management of soilborne pathogens (Lazzeri et al. 2003; Pascual et al. 2004). Glucosinolate hydrolysis products has inspired investigators to explore the use of *Brassicaceae* plant residue for control of different agricultural pests (Mazzola et al. 2007). *Brassicaceae* species can be incorporated into soil as a source of biofumigation

and have shown positive effects on combating soilborne diseases in various field crops (Angus et al., 1994; Matthiessen and Kirkegaard, 2006; Kirkergaard et al. 2000; Yulianti et al. 2006; Brown and Morra 1996; Larkin and Griffin 2007; Matthiessen and Kirkegaard 2006; Smolinska and Horbowicz 1999).

Soil amendment with *Brassica* plants increases organic matter, improves soil structure and boosts beneficial microbial communities as well as their biocidal effects on soilborne pathogens (Larkin and Griffin 2007). Different cultivars of *Brassica* species have different efficacy in biofumigation due to varying concentrations and chemical types of the glucosinolates in their plant tissue (Kirkergaard et al. 1996; Kirkergaard and Sarwar 1998). The degree of fungal suppression by *Brassica* plant materials was determined by the concentration and type of biocidal volatile gases released (Kirkegaard et al. 1996).

Previous *in vitro* bioassays have shown varying effects of *Brassica* plant materials on the suppression of the growth of several plant pathogenic fungi (Smith and Kierkegaard 2002; Sarwar et al. 1998; Charron and Sams 1999; Lazzeri and Manici 2001; Yulianti et al. 2006). However, the *in vitro* bioassays frequently failed to predict the efficacy of *Brassica* biofumigation under field conditions. Such conflict between lab assay and field efficacy may be due to the fact that the methodologies used for screening plant materials for biofumigation exclude the role of soil properties and soil microbes in the process of biofumigants. Gimsing et al. (2006) reported microbial myrosinases in soil influences the *Brassica* biofumigation process, emphasizing the importance of soil properties and microbial activities.

The objective of this study was to screen brassicaceous plant species and other related species as biofumigants for management of rice sheath blight pathogen *R. solani* AG1-IA. The *in vitro* screening method used in this study emulated soil amendment in field conditions of evaluating the antifungal activity of plants tissue and the role of soil properties.

MATERIALS AND METHODS

Soil collection and preparation. Soils used in this study were collected from rice fields in three major rice producing states in the U.S.; Texas (Texas A&M AgriLife Research and Extension Center, Beaumont), Arkansas (Rice Research and Extension Center, Stuttgart), and Mississippi (Delta Research and Extension Center, Stoneville). The soils were sieved using a 2-mm^2 sieve to establish a homogenous soil sample. Soil properties including texture, pH, nutrients and organic content were analyzed at the Soil, Water and Forage Testing Laboratory, Texas A & M AgriLife Extension Service, College Station, TX.

***In vitro* screening.** Nine *Brassica* species and two non-brassicaceous plant species were tested. The brassica species comprised of six mustard (*Brassica juncea*) cvs. Brand 199, Ruby Streak, Florida Broadleaf, Green Wave, Red Giant and Sheali Hong; one turnip (*B. rapa*) cv. Southern Green; one Chinese cabbage (*B. rapa*) cv. Napa; and one kale (*B. oleracea*) cv. Starbor. The two non-brassicaceous plant species were arugula (*Eruca sativa*) cv. Nemat and sunn hemp (*Crotalaria juncea*). The plants were grown in the greenhouse with temperatures ranging from 14 (night) to 36^{o}C (day). At flowering,

the above ground biomass of the plants were harvested and stored in at -20°C until being used for lab assays

Frozen plant material were thawed and macerated with a blender. A 3-g sample of the macerated plant tissue sample of each plant species was confined to the lid of upside down petri dish containing 1/5th strength potato dextrose agar (PDA) medium. An agar disc (8-mm in diameter) of *R. solani* AG 1-IA (TX-RS-1, an isolate collected from a commercial rice field in Texas) was placed on the center of PDA without physical contact with the plant sample attached on the petri dish lid. The petri dishes were incubated at room temperature (25^{o}C) for 72 h and then radial mycelial growth was measured. Relative mycelial growth inhibition of each treatment was calculated by comparing it with the non-treated control. Each treatment consisted of 12 petri dishes per experiment and was arranged in a completely randomized design.

Mixtures of plant tissue and soil were evaluated using the *in vitro* assay method with the experimental protocol and design described above. A macerated plant tissue sample of each plant species was mixed at a 1:1 ratio with each of the soils collected from Texas, Arkansas or Mississippi. The experiment was independently conducted three times.

Effect of brassicaceous plant amendment rate on mycelium growth. Ten grams of Texas soil was placed in an aluminum weighing dish (57 mm in diameter). The soil sample was amended with the *B. juncea* cv. Brand 199 at 0.2, 0.4, 0.8, 1.6 or 3.2% plant tissue (wt/wt), and a non amended control was included. The aluminum dishes containing *B. juncea*-amended soil were placed in a double zipper Ziploc bag (14.9 x

19.6 cm, S.C Johnson and Sons, Racine, WI). Three petri dishes of 1/5th strength PDA, each of which contained a PDA agar disc (8 mm in diameter) of isolate TX-RS-1 of *R. solani* were placed in the bag. Petri dish lids were taken off before the bag was zipped and sealed. The bag containing three *R. solani* culture plates and *B. juncea*-amended soil was incubated at 25ºC. After 48 h of incubation, radial mycelial growth of *R. solani* was measured. Relative mycelium growth inhibition was calculated. The experiment was independently conducted three times.

Effect of soil pasteurization and plant amendment rate on AITC release. The soil from Texas was used to evaluate the effects of soil pasteurization treatment and brassicaceous plant amendment rate on the release of AITC from the *B. juncea* cv. Brand 199. Soil was pasteurized by heating in a dry oven at 60ºC for 18 h. The soil that received no pasteurization treatment served as the natural soil control. A 1-gallon Ziplock bag was filled with 250 g of natural or pasteurized soil was thoroughly mixed with macerated *B. juncea* plant material at a rate of 0.25 or 0.5% (wt/wt). The experiment included five treatments: natural soil with no amendment (non-treated control), natural soil + *B. juncea* amended at a rate of 0.25 or 0.5% (wt/wt), and pasteurized soil + *B. juncea* amended a rate of 0.25 or 0.5% (wt/wt). Each treated soil sample (250 g) was placed in four 0.946-L mason jars assigned in a complete randomization design. Each jar was closed with an air-tight-seal lid containing a rubber septum in the center and incubated at room temperature. To measure the concentration of AITC, 1-ml vapor samples were continually extracted from the head space of each jar by piercing through a rubber septum with a 3.8 cm-long 18-gauge needle (Hamilton Co., Reno, NV) at 12, 24,

36, 48 and 72 h after incubation. After extraction of gases at each time point, the jar lids were briefly removed to release remaining AITC out and then tightly closed back. The vapor samples extracted from continual time intervals were used to measure the pattern of AITC emission from *B. juncea* plant tissue.

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out using a modified protocol of Mazzola et al. (2007). The vapor samples (0.5 ml) were manually injected into an Agilent 7890 gas chromatography (GC) coupled with an Agilent 5975 inert mass spectrometer (MS) (Agilent Technologies, Santa Clara, CA) under a splitless injection mode. The GC was equipped with a HP-5MSI column at 30 m × 0.25 mm I.D., 0.25 μm film thickness. Helium used as a carrier gas was flowed at 2.0 ml/min with an inlet pressure of 120 kPa. The inlet and MS transfer line temperatures were set at 125 and 100°C, respectively. The oven temperature programmed for AITC separation was held at 60°C for 3 min, from 60 to 180°C at a rate of 9°C/min, from 180 to 280°C at a rate of 20°C/min, and finally held at 280°C for 2 min, requiring a total run time of 32.3 min. The MS was operated in scan and selected ion monitoring (SIM) modes for quantification of AITC. MS source temperature was maintained at 230°C, and the mass spectral scanning range 20 to 450 amu (atomic mass unit). Ions of *m/z* 41, 45, 72, and 99 were monitored at retention time 4.0 min for AITC. The standard calibration curve was prepared with a commercial AITC solution (Sigma-Aldrich, St. Louis, MO) to determine the concentrations of AITC. Enhanced Chemstation software (version E.02.01, Agilent Technologies, CA) was used to acquire and process chromatographic peak areas.

Statistical analysis. The data collected from the repetitions of each experiment were combined and subjected to analysis of variance (ANOVA) using SAS 9.2 version (SAS Institute Inc., Cary, NC). Bartlett's test of homogeneity of error variance was carried out prior to combining the data from repetitions of the experiment. Multiple comparisons of significant independent variables were conducted with Fisher's protected least significant difference (LSD) test at $P = 0.05$.

RESULTS

Soil collection and preparation. Soils from Texas and Mississippi were clay soil type whereas the soil from Arkansas was silt loam with higher percentages of sandy and silt (Table 2.1). The soil from Mississippi had the higher levels of pH, conductivity, organic matter, total C, total N, potassium, calcium and magnesium than those from Texas and Arkansas.

Table 2.1. Properties of soils from Texas, Arkansas and Mississippi used in this study.

Soil properties	**Texas**	**Arkansas**	**Mississippi**
Type classification	League clay	Dewitt silt loam	Sharkey clay
Sand (%)	3	18	3
Silt (%)	32	70	32
Clay (%)	64	12	65
pH	6.3	5.8	7.9
Conductivity (umho/cm)	254	139	598
Nitrate-N (mg/kg)	0	27	40
Phosphorus (mg/kg)	10	36	49
Potassium (mg/kg)	168	115	368
Calcium (mg/kg)	3953	827	6745
Magnesium (mg/kg)	637	125	1356
Sulphur (mg/kg)	16	10	18
Sodium (mg/kg)	107	68	62
Total C (mg/kg)	10350	7238	12158
Organic Matter (%)	1.82	1.2	2.24
Total N (mg/kg)	1032	952	1462
Ammonium-N (mg/kg)	12.9	6	2.3

***In vitro* screening of brassicaceous plants.** Plant species, soil type and their interactions were all highly significant ($P \leq 0.01$) in affecting the mycelial growth of *R. solani* (Table 2.2).

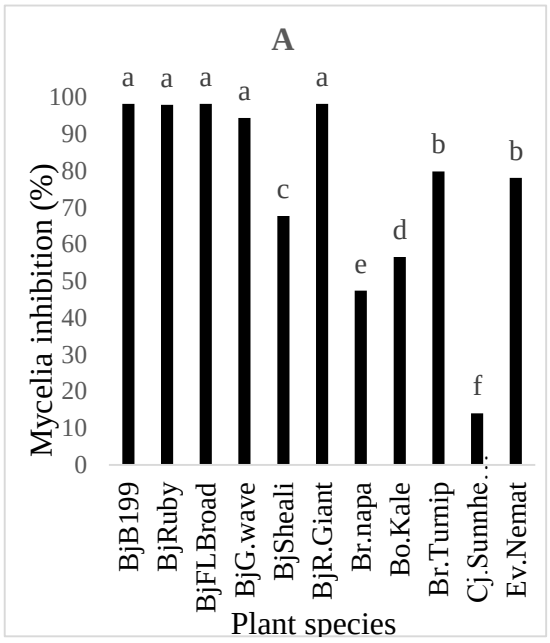
In each of the three soils evaluated, amendments with each of the 11 plants resulted in a significant degree of *R. solani* mycelia growth inhibition (Fig.2.1). All the six mustard (*B. juncea*) cultivars Brand 199, Ruby Streak, Florida Broadleaf, Green Wave, Sheali Hong, and Red Giant consistently inhibited mycelium growth of *R. solani* in three different soil types tested. The cultivars Brand 199, Ruby Streak, Florida Broadleaf and Green Wave (except mixture with Mississippi soil) were the most effective, inhibiting >90% mycelial growth. In the non brassicaceous species tested, arugula (*E. sativa*) cv. Nemat achieved over 60% of *R. solani* mycelia inhibition when amended into the soils from Texas and Arkansas, which was significantly higher than kale (*B. oleracea*), turnip (*B. rapa*) and sunn hemp (*C. juncea*)

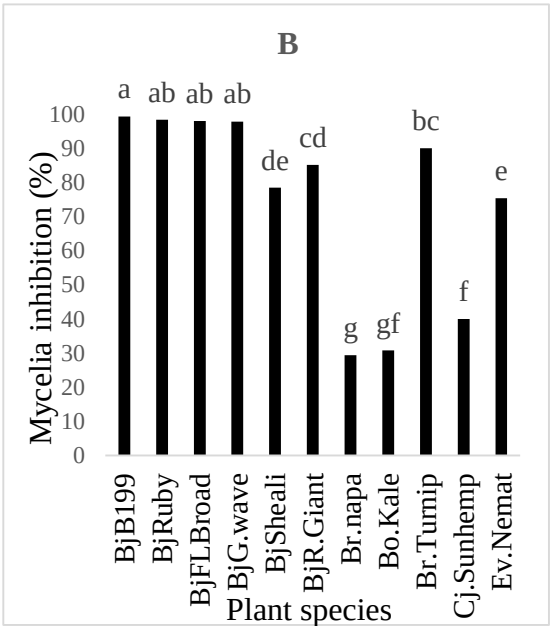
Table 2.2. Analysis of variance for the effect of plant species and soil type on the growth of *Rhizoctonia solani* mycelium.

Source	DF	MS	F-value[z]
Plant species	10	27655	297***
Soil type	3	1343	14***
Plant species x soil type	30	843	9***

[z] ***= Significant at P= 0.0001

Fig. 2.1. Effect of plant species on the inhibition of *Rhizoctonia solani* mycelium. **A**, Amendment to Texas soil; **B**, Amendment to Arkansas soil; **C**, Amendment to Mississippi soil; **D**, No soil used. Treatment abbreviations are as follows: BjB199 = *Brassica juncea* cv. Brand 199; BjRuby = *Brassica juncea* cv. Ruby Streak; BjFLBroad = *Brassica juncea* cv. Florida Broadleaf; BjG.wave = *Brassica juncea* cv. Green Wave; BjSheali = *Brassica juncea* cv. Sheali Hong; BjR.Giant = *Brassica juncea* cv. Red Giant; Br.napa = *Brassica rapa* cv. Napa; Bo.Kale = *Brassica oleracea* cv. Kale Starbor; Br.Turnip = *Brassica rapa* cv. Southern Green; Cj.Sunnhemp = *Crotalaria juncea*; cv.Sunn hemp = *Eruca sativa* cv. Nemat. Bars with the same letter are not significantly different according to Fisher's protected least significant difference at $P = 0.05$.


A
Mycelia inhibition (%)
100
90
80
70
60
50
40
30
20
10
0
a a a a c a e d b f b
BjB199
BjRuby
BjFLBroad
BjG.wave
BjSheali
BjR.Giant
Br.napa
Bo.Kale
Br.Turnip
Cj.Sunnhe…
Ev.Nemat
Plant species


B
Mycelia inhibition (%)
100
90
80
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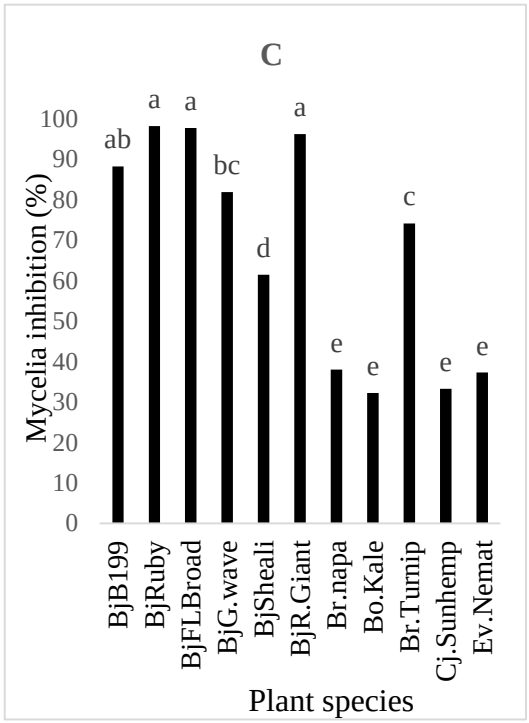
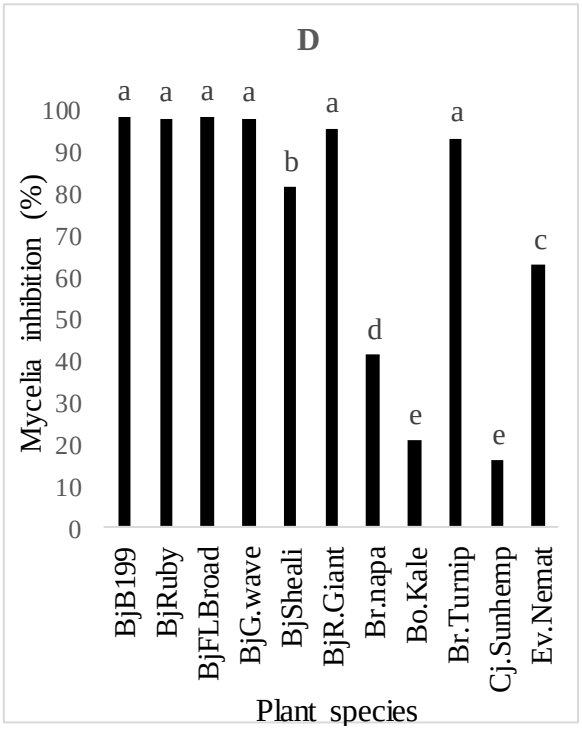
a ab ab ab de cd g gf bc f e
BjB199
BjRuby
BjFLBroad
BjG.wave
BjSheali
BjR.Giant
Br.napa
Bo.Kale
Br.Turnip
Cj.Sunhemp
Ev.Nemat
Plant species


C
Mycelia inhibition (%)
100
90
80
70
60
50
40
30
20
10
0
ab a a bc d a e e c e e
BjB199
BjRuby
BjFLBroad
BjG.wave
BjSheali
BjR.Giant
Br.napa
Bo.Kale
Br.Turnip
Cj.Sunhemp
Ev.Nemat
Plant species


D
Mycelia inhibition (%)
100
90
80
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a a a a b a d e a e c
BjB199
BjRuby
BjFLBroad
BjG.wave
BjSheali
BjR.Giant
Br.napa
Bo.Kale
Br.Turnip
Cj.Sunhemp
Ev.Nemat
Plant species

Soil type significantly affected the efficacy of plant species on inhibiting of mycelial growth (Fig. 2.1). Except for Chinese Napa, kale and sunn hemp, the degrees of mycelial growth suppression by the plant species amended into the soils from Texas or Arkansas were generally greater than those in Mississippi soil. There were no significant differences in the efficacy of mycelial growth inhibition between the soils from Texas and Arkansas

Effect of brassicaceous plant amendment rate on mycelium growth. Mycelia inhibition increased with an increase in the amendment rate regardless of whether the plant tissue was amended with or without soil (Fig. 2.2). Plant amendment rate, soil use and their interactions were significant ($P \leq 0.01$) in affecting the mycelial growth of *R. solani* (Table 2.3). Amendment rate of the *B. juncea* cv. Brand 199 accounted for 87% of the variation in *R. solani* mycelium inhibition. Plant tissue without soil tended to achieve higher levels of mycelium inhibition of *R. solani* than plant tissue amended with soil.

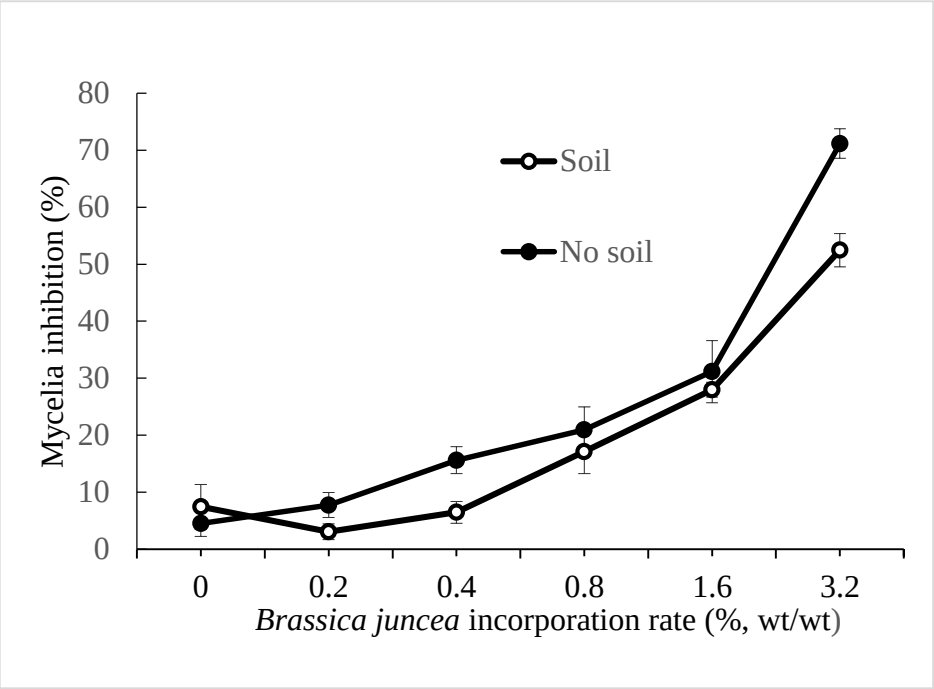

Fig. 2.2. Effect of *Brassica juncea* plant tissue amendment rate on *Rhizoctonia solani* mycelium growth when mixed with Texas soil or without soil. Bars represent ± standard deviation of the mean. Error bars are hidden when smaller than the symbols.

Table 2.3. Analysis of variance for the effect of soil medium and *Brassica juncea* application rate on the growth of *Rhizoctonia solani* mycelium.

SOV	DF	MS	F-value [z]
Soil medium [y]	1	1156.2	16.58***
Rate	5	9095	130.42***
Media x Rate	5	197	2.83**

[y] Medium refers to whether *Brassica juncea* plant tissue was mixed with soil or no soil

[z] **= Significant at P= 0.01; *** = Significant at P=0.0001

Effect of soil pasteurization and plant amendment rate on AITC release. Soil pasteurization, *B. juncea* plant amendment rate, time of sampling and their interactions were highly significant ($P \leq 0.01$) in affecting the amount of AITC emitted from plant tissue of *B. juncea* cv. Brand 1999 (Table 2.4).Plant tissue amendment in natural (non-pasteurized) soil resulted in a significantly higher AITC release than plant tissue amendment in pasteurized soil (Fig. 2.3 and 2.4). In natural soil, emission of AITC reached higher levels from the soil amended with 5% than 2.5% (wt/wt) of *B. juncea* (Fig. 2.4). The soil amended with 5% (wt/wt) of *B. juncea* released 96% more AITC than the soil amended with 2.5% (wt/wt) of *B. juncea*. The highest and lowest AITC levels from the amendment treatment of 5% (wt/wt) *B. juncea* were recorded at 24 and 36h after amendment, respectively. In pasteurized soil, AITC amounts increased from 12 to 24 h and then leveled off from 36 to 72 h regardless of the amendment rate of *B. juncea* evaluated (Fig. 2.4).

Table 2.4. Analysis of variance for the effect of soil pasteurization treatment, *Brassica juncea* application rate and sampling time emission of allyl isothiocyanates.

SOV	DF	MS	F-value [z]
Soil pasteurization treatment (S)	1	24.5	33.0***
Application rate (R)	1	25.8	34.7***
Time (T)	4	10.9	3.6**
S x R	1	21	28.2***
S x T	4	8.7	2.9**
R x T	4	11.4	3.8**
S x R x T	4	10.1	3.3**

[z] **= Significant at P= 0.01; *** = Significant at P=0.0001

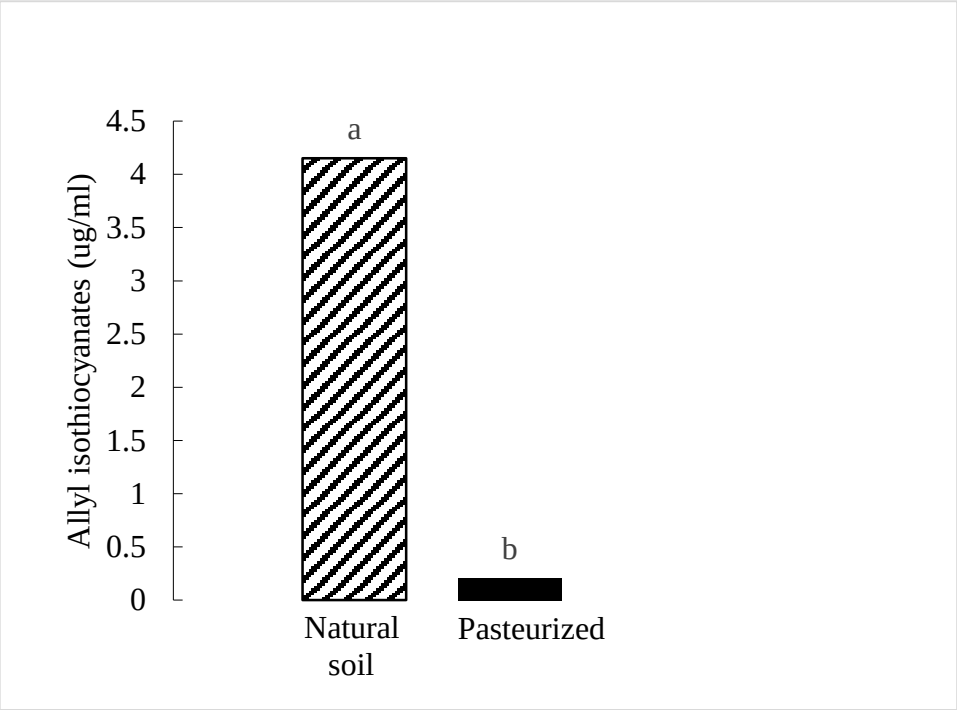

Fig. 2.3. Cumulative allyl isothiocyanates emitted from natural and pasteurized soils.

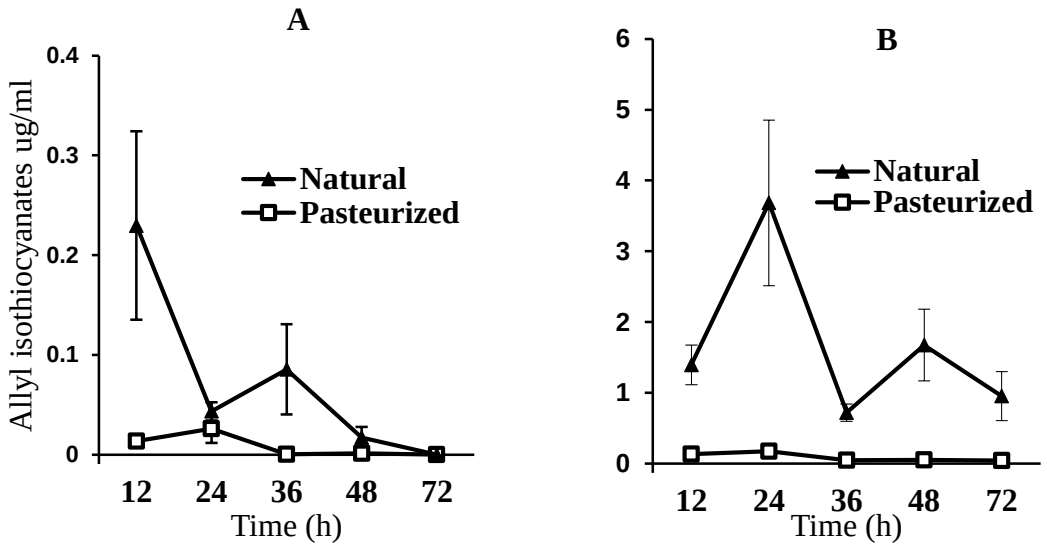

Fig. 2.4. Time-course emission of ally isothiocyanate from natural or pasteurized Texas soil amended with 0.25% (**A**) and 0.5% (**B**) (wt/wt) *Brassica juncea* plant tissue. Bars represent ± standard deviation of the mean. Error bars are hidden when smaller than the symbols.

DISCUSSION

Effective mycelium inhibition by *B. juncea* plant species tested in this study can be attributed to high levels of glucosinolates in their plant tissue as previously reported by Mazzola and Zhao (2010). Variation in efficacy of *Brassica*ceae plants for inhibiting *R. solani* mycelium growth in our *in vitro* assay is associated with glucosinolate content. Different *Brassica* species or cultivars have different concentrations and types of the glucosinolates (Kirkergaard et al. 1996; Kirkergaard and Sarwar 1998). In this study, *B. juncea* species evaluated in this study outperformed other species in antifungal activity because they contained higher levels of allyl glucosinolate type, resulting in the release of higher concentrations of AITCs upon hydrolysis. The AITCs released from macerated *B. juncea* tissue has been shown to have strong antimicrobial activities inducing *Rhizoctonia solani* (Mayton et al. 1996). The efficacy of mycelium growth inhibition by sunn hemp (*C. juncea*) and arugula (*E. sativa*) could be attributed to a different chemistry (Lazzeri et al. 2003, Sharma et al. 2003).

Soil properties is another important factor that determines the efficacy of biofumigation because it directly affect hydrolysis of glucosionolates and subsequent release of AITC from brassicaceous plant tissue (Brown and Morra 1996; Tsao et al. 2000). Our *in vitro* study employed the treatments of mixing *B. juncea* plant tissue with soil mimicking field conditions with the presence of soil microbes. Mycelial growth inhibition resulting from different *B. juncea* amendments seem to be consistent in the soils from Texas and Arkansas in contrast to Mississippi. These differences might be associated with the differences in soil properties such as pH, organic matter and total N.

Most soil properties were similar in the soils from Texas, Arkansas and dissimilar from Mississippi soil. These findings indicate that soil texture does not affect the efficacy of *B. juncea* amendment but the other soil properties contribute to biofumigation efficacy. More detailed research is needed to understand the impacts of pH, organic matter, total N and other soil properties on the release of AITC and microbial activities.

Biofumigation is dose dependent. As the amount of *B. juncea* plant tissue amended into soil increases, AITC emission increases and consequently antifungal activity becomes stronger. Disease suppression is achieved in response to chemistry-based mode of action depending upon the efficiency at which glucosinolate are converted into isothiocyanate (Mazzola and Zhao 2010). Our *in vitro* assay shows a positive correlation among antifungal activity, AITC emission and the amount of glucosinolate *B. juncea* plant tissue. This supports a major role of glucosinolates in the biofumigation process, indicating that total glucosinolate contents in biofumigants are a primary factor in *in vitro* screening and biofumigation efficacy.

Release of AITC appears to be very rapid within 12 to 24 h after treatment and then decreased to undetectable levels after 72h regardless of the amount of *B. juncea* tissue used. In a previous relevant study by (Mazzola and Zhao 2010), AITC emission from *B. juncea* amended soil was completed within 72 h post amendment, even at an application rate to soil up to 1.0 % (wt/wt). Morra and Kirkegaard (2002) reported that maximum AITC release in the field occurred at 2 h after *Brassica* tissue incorporation. In this study, most of the AITC were released and depleted within the first 4 days after tissue incorporation.

The lower AITC emission observed in the pasteurized soils compared to the natural soils are attributed to a reduction of microbial or enzymatic activities by soil pasteurization. Previous studies particularly focused on the importance of myrosinase in the biofumigation process. Soil sterilization resulted in a significant reduction of myrosinase activity (Gimsing, et al. 2006). The rapid dissipation of glucosinolates in brassicaceous plant materials amended in the natural soil is attributed mainly to myrosinase activity (Omirou et al. 2013). The reduction of AITC emission in pasteurized soils observed in this study suggests that activities of soil microbes play an important role in glucosinolate hydrolysis.

CONCLUSIONS

Results of this study demonstrate antifungal property of *B. juncea* plants, especially cultivar Brand 199, as a biofumigation candidate to manage rice sheath blight caused by *R. solani* AG1-1A. Incorporation of *B. juncea* plant tissue can also improve soil microbial activities and soil abiotic properties suppressive to sheath blight. Both biotic and abiotic factors should be considered for improving the antifungal efficacy when screening for brassicaceous biofumigants and implementing biofumigation practices to manage soilborne disease in the field.

CHAPTER III

SOIL AMENDMENT WITH *BRASSICA JUNCEA* REDUCES VIABILITY, AGGRESSIVENESS, AND SCLEROTIA FORMATION OF *RHIZOCTONIA SOLANI* AG1-1A

OVERVIEW

Brassica juncea plant materials are used as a soil biofumigant for control of soil-borne pathogens. Biocidal effects have been attributed to volatile ally isothiocyanates after hydrolysis of glucosinolates contained in *B. juncea*. *In vitro* inhibition of the mycelium growth of *Rhizoctonia solani* by *B. juncea* is well documented. The objective of this study was to determine the impacts of *B. juncea* as soil amendment on the viability, aggressiveness, and sclerotia formation of *R. solani* AG1-1A, the causal agent of sheath blight in rice. In laboratory experiments, the effects of *B. juncea* amendment rate (0, 0.2, 0.4, 0.8, 1.6 or 3.2, wt/wt) and duration of exposure (7, 14, or 28 days) on *R. solani* AG1-1A were evaluated. Significantly reduced numbers of sclerotia were formed from the mycelium exposed to soils amended with *B. juncea* at 0.2 to 3.2% at 7, 14 or 28 days of exposure when compared to the mycelium exposed to the non-amended soil. The viability of sclerotia after exposure for 14 and 28 days to soils amended with 1.6 and 3.2 % of *B. juncea* was significantly lower than that of sclerotia exposed to non-amended soil. Sclerotia or mycelium after exposure for 28 days to soil amended with 3.2 % of *B. juncea* resulted in a reduction in aggressiveness, showing shorter sheath blight lesion length on detached rice leaves than the non-treated controls. Results of this study demonstrate for the first time that *B. juncea* soil amendment can reduce the viability and

aggressiveness of *R. solani* mycelium and sclerotia and affect the formation of sclerotia. Soil amendment with *B. juncea* plant tissue can provide an effective means to reduce viability and aggressiveness of the primary inoculum of sheath blight in rice.

INTRODUCTION

Rhizoctonia solani Kühn AG1-1A [teleomorph *Thanatephorus cucumeris* (A.B. Frank)] is a ubiquitous soil-borne fungus that causes sheath blight in rice. Sheath blight is one of the most devastating diseases in rice and has become a major threat to worldwide rice production (Groth et al. 2003). Commercial rice cultivars have no major genes conferring dominant resistance to sheath blight, and therefore sheath blight management is largely dependent on fungicide applications (Groth et al. 2003). Excessive use of fungicides increases production costs and poses potential risks to the human health and the environment. Rice is often rotated with other crops to reduce sheath blight. However, a broad host range of *R. solani* leaves most farmers with no option but use susceptible crops such as soybean in their crop rotations. There is a need to develop alternative management strategies for sheath blight of rice that are more sustainable and environmentally friendly.

Biofumigation is one of alternative biological approaches for disease management, which uses biocidal compounds produced during the breakdown of brassicaceous plant materials in soil. A number of studies have demonstrated the potential use of brassicaceous biofumigants to control soilborne plant pathogens and diseases such as *Rhizoctonia* spp. and take-all diseases of wheat (Murray and Brenna

1988; Charron and Sams 1999), nematodes and weeds (Matthiessen and Kirkegaard 2006). However, inconsistencies in the effectiveness of brassicaceous biofumigation have been also reported in some crop systems (Njoroge et al. 2008; Motisi et al. 2009; Motisi et al. 2010). This is, in part, due to differences in sensitivity of propagules or survival forms of soilborne pathogens to biofumigants. The tolerance of the resting structures of pathogens to the allyl isothiocyanates (AITCs) released from *Brassica* plant tissue is related to the failure of biofumigation in disease suppression (Yulianti et al. 2008). Therefore, to be effective for control of soilborne diseases, brassicaceous plants must contain the levels of glucosinolates high enough to affect the resting or overwintering structures of pathogens. It has been shown that *Brassica juncea* plant tissue releases effective levels of glucosinolates only when its above ground biomass reaches 5.7 kg m^{-2} or more (Antonious et al. 2009).

Previous studies have focused on evaluating the *in vitro* effects of AITCs released from macerated *B. juncea* plant tissue on the growth of mycelium of plant pathogenic fungi including *R. solani* (Mayton et al. 1996; Yulianti 2008; Zhou et al. 2011). There is a dearth of information on the impacts of *B. juncea* soil amendment on the mycelium and sclerotia of *R. solani* AG1-1A, which is crucial to the development of an effective brassicaceous biofumigation approach for management of sheath blight in rice.

The objective of this study was to evaluate the effects of amendment rate and exposure time of *B. juncea* soil amendment on the viability and aggressiveness of mycelium and sclerotia and on the formation of sclerotia of *R. solani* AG1-1A.

MATERIALS AND METHODS

Mycelium aggressiveness and sclerotia formation after exposure to soils amended with *B. juncea* plant tissue. Experiments were conducted using a sieved League type soil (3% sand, 32% silt and 64% clay) collected from rice fields at the Texas A&M AgriLife Research and Extension Center in Beaumont, TX. Ten grams of sieved soil were placed in an aluminum weighing dish (57 mm in diameter). Each soil sample was amended with *B. juncea* at 0.2, 0.4, 0.8, 1.6 or 3.2% (wt/wt), and a non-amended control was included. The aluminum dishes containing *B. juncea*-amended soil were placed in a double zipper Ziploc bag (14.9 x 19.6 cm, S.C Johnson and Sons, Racine, WI). Two 84-mm petri dishes containing 3-day-old mycelium of *R. solani* AG1-1A growing on potato dextrose agar (PDA) medium were placed in each bag, and lids taken off before the bags were zipped and sealed. A total of eight petri dishes of *R. solani* cultures were used for each treatment. Each treatment was in a completely randomized design with four replications.

The bags containing *R. solani* cultures and soils amended with *B. juncea* plant tissue were incubated at 25 ºC. After 7, 14 or 28 days of incubation, the bags were opened, and aluminum dishes carrying soil amended with *B. juncea* plant tissue were removed and discarded. Five PDA discs (8 mm in diameter) with *R. solani* mycelium were taken out from each petri dish and individually placed at the center (lengthwise) of a 5-cm long, 8-week-old rice leaf blade (cv. Presidio, susceptible to sheath blight) laid in a petri dishes lined with moistened filter paper at the bottom. The petri dishes were incubated at 25ºC for 72 h, and the length of sheath blight lesions developing on the

leaves was measured. The original *R. solani* cultures were returned into respective empty bags after removing mycelial discs, and then the bags were zipped and kept at 25ºC. The number of sclerotia formed on mycelium was counted at 30 days after the initiation of the experiment. The experiment was independently conducted three times.

Sclerotia viability and aggressiveness after exposure to soils amended with *B. juncea* plant tissue. A 250-g sieved League type soil sample was placed in each 0.5-L Mason jar and thoroughly mixed with macerated *B. juncea* plant tissue at the rates of 0.2, 0.4, 0.8, 1.6 or 3.2% (wt/wt). A non-amendment soil treatment was included as a control. Fifteen 10-day-old sclerotia (approximately 2 mm in diameter) of *R. solani* AG1-1A were wrapped with a gauze pad and bound on the inside of each Mason jar lid that was used to close the jar containing *B. juncea* soil amendment. Each soil amendment treatment consisted of eight Mason jars and was arranged in a completely randomized design. After incubation at 25ºC for 7, 14 and 28 days, Mason jar lids were taken off and sclerotia were removed off the lids. The sclerotia were surface sterilized by immersing in l0% sodium hypochlorite for 5 minutes and followed by rinsing with sterile water for 5 minutes. After air drying, five sclerotia were individually placed at the center of rice leaf blades, and lesion development was measured as the aforementioned method. The remaining 10 sclerotia were evaluated for viability by placing them on 1/5th strength PDA plates. Germinated sclerotia were counted after 96 h of incubation at 25ºC. The viability of sclerotia was calculated as the percentage of sclerotia that germinated. The experiment was independently conducted three times.

Statistical analysis. The data collected from the three repetitions of each experiment were combined and subjected to analysis of variance (ANOVA) using SAS 9.2 version (SAS Institute Inc., Cary, NC). Bartlett's test of homogeneity of error variance was carried out prior to combining the data from three repetitions of each experiment. Multiple comparisons of significant independent variables were conducted with Fisher's least significant difference (LSD) test at $P = 0.05$.

RESULTS

Brassica juncea application rate, exposure duration and their interaction significantly ($P \leq 0.05$) affected mycelium in producing sclerotia in 30 days and sheath blight lesion on rice leaves (Table 3.1). Lower numbers of *R. solani* sclerotia were produced from the mycelium exposed to soils amended with *B. juncea* at 0.2 to 3.2% for 7, 14 or 28 days when compared to the mycelium exposed to the non-amended soil control (Table 3.2). Significant reductions in sheath blight lesion length were observed from the mycelium that was exposed to soil amended with *B. juncea* plant tissue at the rates of 0.8, 1.6 or 3.2% for 7 and 14 days as well as at the rates of 0.4, 1.6 or 3.2% for 28 days (Table 3.2).

Table 3.1. Analysis of variance for the effects of application rate and exposure time of *Brassica juncea* soil amendment on sclerotia formation and aggressiveness (sheath blight lesion length on rice leaves) of *Rhizoctonia solani* mycelium.

		No. of sclerotia		Lesion length	
Source	DF	MS	*F*-value	MS	*F*-value
Rate (R)	5	31339	31.9***	1.6	4.6***
Exposure time (T)	2	3768	3.8*	2.7	7.7***
R x T	10	4642	4.7***	2.4	1.5***

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; all other mean squares were not significant.

Table 3.2. Effects of the interactions between application rate and exposure time of *Brassica juncea* soil amendment on sclerotia formation and aggressiveness (sheath blight lesion length on rice leaves) of *Rhizoctonia solani* mycelium.

	No. of sclerotia (per petri dish)			Lesion length (cm)		
Application rate (%, wt/wt)	7 days	14 days	28 days	7 days	14 days	28 days
0	180 a[z]	180 a	145 a	3.6 a[y]	4.0 a	4.0 a
0.2	91 b	120 b	137 ab	3.3 ab	3.4 ab	3.9 a
0.4	75 bc	98 bc	105 bc	3.3 ab	3.8 a	3.4 b
0.8	82 bc	84 cd	95 cd	2.8 bc	2.9 bc	3.7 ab
1.6	60 c	69 cd	51 e	2.8 bc	2.7 c	3.0 c
3.2	28 d	64 d	67 de	2.4 c	2.6 c	2.8 c

[z] Means in the same column with the same letter are not significantly different at $P = 0.05$.

Brassica juncea application rate, exposure duration and their interaction significantly ($P \leq 0.05$) affected sclerotia in their viability and sheath blight lesion development on rice leaves (Table 3.3). Sclerotia viability after exposure to soils amended with 1.6 and 3.2 % of *B. juncea* for 14 and 28 days was significantly lowered compared to the non-amended soil control (Table 3.4). After exposed to soil amended with 0.4% of *B. juncea* for 7 days or exposed to soil amended with 3.2 % for 28 days, sclerotia produced a significantly lower lesion length than those exposed to the non-amended soil control (Table 3.4). The other treatments with different combinations of application rate and exposure time of *B. juncea* did not reduce lesion development significantly.

Table 3.3. Analysis of variance for the effects of application rate and time of exposure of *Brassica juncea* soil amendment on the viability and aggressiveness (sheath blight lesion length on rice leaves) of *Rhizoctonia solani* sclerotia.

		Viability		Lesion length	
Source	DF	MS	*F*-value	MS	*F*-value
Rate (R)	5	598	5.2**	0.9	2.2**
Exposure time (T)	2	5121	44.9***	17.1	40.8***
R x T	10	370	3.2*	2.1	5.1***

* = $P \leq 0.05$; ** = $P \leq 0.01$; ***= $P \leq 0.001$; all other mean squares were not significant.

Table 3.4. Effects of the interactions between application rate and exposure time of *Brassica juncea* soil amendment on the viability and aggressiveness (sheath blight lesion length on rice leaves) of *Rhizoctonia solani* sclerotia.

	Viability (%)			Lesion length (cm)		
Application rate (%, wt/wt)	7 days	14 days	28 days	7 days	14 days	28 days
0	93 ab[z]	98 a	78 a	3.1 abc	4.1 a	1.6 a
0.2	100 a	100 a	47 bc	2.4 cd	3.5 a	3.1 a
0.4	87 ab	93 ab	80 a	2.1 d	4.3 a	2.0 a
0.8	80 b	87 abc	62 ab	3.3 ab	4.3 a	2.0 a
1.6	80 b	75 c	55 bc	2.8 bcd	4.0 a	2.8 a
3.2	93 ab	83 bc	37 c	3.7 a	3.1 a	0.2 b

[z] Means in the same column with the same letter are not significantly different at $P = 0.05$

DISCUSSION

Results of this study demonstrate for the first time that *B. juncea* soil amendment can reduce the viability and aggressiveness of mycelium and sclerotia and affect the formation of sclerotia of *R. solani* AG1-1A. This information will be helpful to develop and facilitate the use of brassicaceous biofumigation for management of sheath blight in rice.

Sensitivities of *R. solani* AG1-1A to *B. juncea* volatile compounds varied depending on application rate, exposure duration and type of propagules. Amount of *B. juncea* plant tissue used for soil amendment was positively correlated to the reductions in viability and aggressiveness of *R. solani* AG1-1A. Similar relationships were previously reported between *B. juncea* amendment rate and *in vitro* hyphal growth inhibition of the other *R. solani* AG groups (Charron and Sams 1999; Mayton et al., 1996). However, the two propagule types, mycelium and sclerotia, of *R. solani* AG1-1A responded differently to *B. juncea* in viability and aggressiveness. Compared to mycelium, reduced sensitivity of sclerotia to *B. juncea* may be explained by that AITCs released from *B. juncea* failed to penetrate through sclerotia. Sclerotia of *R. solani* are compact masses of hardened and melanized mycelium, which serve as primary inoculum in soil (Jacobson 2000). Therefore, sclerotia are relatively less sensitive to biocidal volatiles released from *B. juncea* or pure AITC compounds compared to mycelium (Yulianti et al. 2006). In the current study, decreased viability of *R. solani* AG1-1A sclerotia was only observed at the longer durations of exposure (14 or 28 days) with high levels of *B. juncea* amendment (1.6 or 3.2 %, wt/wt). Reduced aggressiveness of sclerotia on rice leaves was also

observed only at a higher amendment rate (3.2%) of *B. juncea* with a longer period of exposure (14 and 28 days). Similarly, Yulianti et al. (2006) previously reported different sensitivities between mycelium and sclerotia of *Rhizoctonia solani* AG 2-1.

Various glucosinolate hydrolytic products released from *B. juncea* have a significant role in disease suppression (Mazzola et al. 2007). The current study only focused on volatile compounds of *B. juncea* that were evaluated since there was no direct contact between the *B. juncea* plant tissue-amended soil and *R. solani* AG1-1A inoculum. Volatile compounds of glucosinolate hydrolytic products played important roles involved in the suppression of the viability and aggressiveness of sclerotia and mycelium of *R. solani* AG1-1A. The efficacy of *B. juncea* soil amendment seems to be dependent on the AITCs released from *B. juncea* since its efficacy was much more consistent at a higher amendment rate and a longer period of exposure.

CONCLUSIONS

Not only did *B. juncea* soil amendment reduce the aggressiveness of *R. solani* AG1-1A mycelium, but also reduced the aggressiveness of sclerotia. This implies that proper use of *B. juncea* soil amendment before rice planting has potential to reduce the amount of effective primary inoculum. Since sheath blight is a monocyclic disease, *B. juncea* biofumigation used to reduce primary inoculum in the forms of sclerotia or mycelium would be much effective in reducing sheath blight development in a rice-cropping season. More investigation is needed to validate the efficacy of *B. juncea* biofumigation approach for management of sheath blight in rice under field conditions.

CHAPTER IV*

INTEGRATION OF *BRASSICA* COVER CROP WITH HOST RESISTANCE AND AZOXYSTROBIN FOR MANAGEMENT OF RICE SHEATH BLIGHT

OVERVIEW

Sheath blight caused by *Rhizoctonia solani* is the most important rice disease that can cause significant losses in grain yield and quality in the southern United States. Current management options for sheath blight primarily consist of fungicides, tolerant cultivars and cultural practices. These options are not always very effective. *Brassica* plants have been used for soil fumigation to manage a variety of different soil-borne pathogens. In this field study, the efficacy of a *Brassica juncea* cover crop, integrated with use of a tolerant rice cultivar and fungicide application was evaluated in 2011, 2012 and 2013. The *B. juncea* cover crop significantly lowered sheath blight severity in all three years and led to a significantly higher grain yield in 2013 as compared to the fallow control. Rice cultivar 'Presidio' had lower sheath blight severity and higher yield than 'Cocodrie' in 2012 and 2013. Fungicide applications with azoxystrobin at the label rate (0.16 kg a.i./ha) or half the label rate (0.08 kg a.i./ha) significantly reduced sheath blight severity in all the three years, resulting in a yield increase in two of the three years. *B.*

* Handiseni, M., Jo, Y.-K., and Zhou, X.-G. Integration of *Brassica* cover crop with host resistance and azoxystrobin for management of rice sheath blight. Plant Dis. In Press. DOI: 10.1094/PDIS-08-14-0845-RE

juncea along with use of a tolerant rice cultivar and half the label rate of azoxystrobin can be an effective approach for management of sheath blight in rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in fighting against global hunger. Over half of the world's population depend on rice as their staple diet. The United States rice industry produced more than 958, 000 tons from more than 997, 000 ha in 2013 (USDA 2014). One of the major diseases limiting rice production in the southern United States is sheath blight, caused by *Rhizoctonia solani* Kühn AG1-1A [teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk] (Groth and Lee 2003). Sheath blight can cause as much as 50% yield loss and significant loss in grain quality (Groth 2005). The pathogen overwinters in infected plant tissue as sclerotia and mycelium, which serve as the primary inoculum and may survive in the soil for up to two years (Groth 2005). It has a wide host range comprised of both monocots and dicots (Larkin and Griffin 2007) including soybean. Many rice farmers in the United States rotate rice and soybean, both of which serve as hosts for *R. solani*. There has been an increase in sheath blight incidence and severity, and this has been attributed to modern rice production practices such as large-scale monoculture, use of semi-dwarf cultivars, high nitrogen fertilization, and high plant populations (Groth and Lee 2003). There are no commercial rice cultivars highly resistant to sheath blight. Therefore, sheath blight management practices have been focused on the use of tolerant varieties, optimized nitrogen fertility, proper seeding rates and inoculum-reducing practices such as rotation, cultivation and sanitation (Groth

and Lee 2003;Groth 2005; Kumar et al. 2009). Given the application of these cultural disease management practices, rice farmers in the United States still heavily rely on synthetic fungicides. Intensified and large-scale use of fungicides can result in negative impacts on the environment (Bunemann et al. 2006; Gullino and Kuijpers 1994).

Biofumigation using *Brassica* plant materials has grown as an environmentally friendly approach to plant pest management (Mazzola et al. 2007). The pesticidal effect of the *Brassica* plant is based on the production of toxic and volatile isothiocyanates upon hydrolysis by myrosinases. Isothiocyanates have biocidal activity toward numerous plant pests (Brown and Morra 1996), parasitic nematodes (Subbarao et al. 1999; Henderson et al. 2009; Izzo and Mazzola 2006), and plant pathogenic fungi and oomycetes (Manici et al. 1997; Mazzola et al. 2007). Efficacy of *Brassica* plant tissue has been reported against verticillium wilt (*Verticillium dahlia*) on cauliflower (*Brassica oleracea* var. *botrytis*) (Subbarao et al. 1999), *Sclerotinia sclerotiorum* (Smolinska and Horbowicz 1999), *Verticillium dahliae* (Oliver et al. 1999), take-all (*Gaeumannomyces graminis*) on wheat (*Triticum aestivum*) (Kirkegaard et al. 2000), and Sclerotinia drop (*Sclerotinia minor*) on lettuce (*Lactuca sativa*) (Hao et al. 2003). Techniques such as use of *Brassica* seed meals and macerated *Brassica* plant tissue have been developed to achieve soil fumigation. Biofumigation was reported to be effective for suppressing *R. solani* infection in apple (*Malus sylvestris* (L.) Mill.) cropping systems (Mazzola et al. 2007). *B. juncea* cover cropping and subsequent plant tissue incorporation into the soil prior to rice planting may be of great merit as a sustainable management option for control of sheath blight, but has not, to our knowledge, been evaluated in the field. The objective of this

study was to evaluate efficacy of integrating a *B. juncea* cover crop with the use of a tolerant cultivar and application of azoxystrobin on rice sheath blight.

MATERIALS AND METHODS

Experimental location and design. Experiments were conducted from 2011to 2013 in rice fields having League type soil (3% sand, 32% silt and 64% clay) at the Texas A&M AgriLife Research and Extension Center in Beaumont, Texas. The experimental area was changed each year and the plots were newly established. In 2011, the rice cultivar 'Cocodrie', susceptible to sheath blight, was used. The experiment was conducted as a split-plot design with four replications. Plots consisted of seven 5.5 m long rows with a spacing of 20 cm between rows. The main plots consisted of cover crop treatment with *B. juncea* cultivar 'Caliente 199' or non-treated fallow. The sub plots were assigned with azoxystrobin application at the rate of 0, 0.08 (half the label rate) and 0.16 kg (the label rate) active ingredient (a.i.) ha^{-1}. In 2012 and 2013, the experiment was conducted as a split-split-plot design with four replications. Two rice cultivars 'Cocodrie' and 'Presidio', susceptible and tolerant to sheath blight, respectively, were used as a split effect to the main plot effect of cover crop treatment. The sub-sub plots were three levels of fungicide application rate as described above.

Inoculation. Autoclavable polypropylene plastic trays (16 liters in volume) (BEI Art Products, Wayne, NJ) containing rice grain and hull mixture (1:2 vol/vol) were filled to one-fifth distilled capacity with distilled water (vol/vol), covered with aluminum foil and autoclaved for 30 min at 121°C. The autoclaved grain mixture was kept covered for

24 h at room temperature (~25^{o}C), and then autoclaved for a second time and allowed to cool. The double-autoclaved grain mixture was then inoculated with the isolate TX-RS-1 of *R. solani* AG1-1A that was cultured on 1/5-strength potato dextrose agar (PDA) for 4 days. TX-RS-1 was isolated from a rice plant showing symptoms of sheath blight in a commercial field in Texas. The inoculated grain mixture was covered with aluminum foil and incubated at room temperature for 4 wks. During the incubation period, the inoculated grain-hull mixture was thoroughly mixed under aseptic conditions in the fume hood every 7 days to allow uniform colonization by *R. solani* mycelium. The colonized grain-hull inoculum was air dried at room temperature for 5 days and stored at room temperature before use. Each plot was inoculated with *R. solani* by manually broadcasting 1.2 liters of *R. solani*-colonized grain inoculum, one month prior to seeding of *B. juncea* cover crop.

Field plots. At the first-season field trial, *B. juncea* 'Caliente 199' was seeded at 5.6 kg/ha on 25 March 2011. To determine the amount of *B. juncea* aboveground biomass dry weight, a 1 x 1 m quadrant was placed randomly at three different spots in the field and the *B. juncea* biomass in the quadrant area was cut at soil level, oven dried and weighed. The *B. juncea* plants (3,451 kg/ha) were plowed down and incorporated into the soil at the flowering stage on 2 June, 2011. Rice was drill seeded at 89.6 kg/ha on 14 June, 2011. Rice plots were treated with 63.8 N kg/ha at post emergence on 20 June, and experienced permanent flood on 11 July and panicle differentiation on 4 August in equal installments. Control of weeds and insect pests and irrigation followed the Texas rice production guidelines (Way et al. 2013). At the boot stage, plots were sprayed with azoxystrobin (Quadris 2.08 SC, Syngenta, Raleigh, NC) at 0, 0.08 (half of the label rate)

and 0.16 (the label rate) kg a.i./ha using a CO_2 pressurized sprayer equipped with a boom of three Tee Jet 8002 nozzles spaced 0.4m apart that delivered at 299 liter/ha.

At the second-season field trial, *B. juncea* 'Caliente 199' was seeded at 5.6 kg/ha on 7 October 2011 and its aboveground biomass (5,446 kg/ha) was incorporated into the soil on 3 January 2012. Rice was drill seeded on 28 March. For the third-season field trial, sowing of *B. juncea* 'Caliente 199' was done on 12 October 2012 and its aboveground biomass (5,974 kg/ha) was incorporated into the soil on 6 March 2013. Rice was seeded on 13 April. At the second and third seasons, permanent flood in the rice field were established in late May. Fertilizer, insect pest and weed control management were done following the Texas rice production guidelines (Way et al. 2013).

Data collection and analysis. Severity of sheath blight was assessed using a scale of 0 to 9, where 0 = plants healthy, no symptoms; 1 = restricted dark brown oval lesions at water line or infection points; 2 = few oval or coalesced lesions with broad borders on lower sheaths or at infection points, 5% or less of tissue affected; 3 = lesions on lower leaf sheaths or at infection points, lesions coalescing, less than 10% of tissues affected; 4 = lesions mainly restricted to sheaths on lower third of plant, lowest leaves, or other infection points, lesions discrete or coalescing with narrow red-brown border, 10 to 15% of leaf and sheath tissues affected; 5 = lesions mainly restricted to sheaths and leaves of lower half of plants, lesions usually coalescing with large necrotic centers and narrow red-brown borders, 15 to 25% of tissues affected; 6 = lesions usually coalescing and affecting lower two-thirds of sheath area of plant, lesions extending to blades of lower leaves or lower leaves killed by injury to sheath, 25 to 40% of tissues affected; 7 =

lesions usually coalescing and affecting lower three-fourths of sheath area of plant, lesions extending to leaf blades of lower two-thirds of plant, 40 to 60% of tissues affected; 8 = lesions reaching to flag leaf, lower sheaths with coalesced lesions covering most of tissue, lower and middle leaves dead or dying, 60 to 80% of tissues affected; and 9 = lesions reaching to flag leaf, lower leaves mostly dead, sheaths dried, culms brown, collapsing, most tillers lodged, over 80% of tissue affected (Groth 2005). Disease severity was measured on 21 September 2011, 21 August 2012 and 1 August 2013, approximately one week before harvesting. Plots were harvested at maturity using a plot combine each year. Grain moisture contents were determined, and rice yields were adjusted to 12% grain moisture content. The data were subjected to analysis of variance (ANOVA) using SAS statistical software (Version 9.2, SAS Institute, Cary, NC) to determine treatment effects. Multiple comparisons of significant independent variables were conducted with the Fisher's least significant difference (LSD) test.

RESULTS

Brassica juncea cover crop significantly ($P < 0.05$) reduced sheath blight severity consistently in all three years tested (Table 4.1), and resulted in a significant yield increase in 2013 compared with the fallow control (Table 4.2). Cultivar 'Presidio' had significantly less sheath blight severity and produced higher grain yields than 'Cocodrie' in 2012 and 2013 (Table 4.3). Azoxystrobin application significantly ($P < 0.05$) reduced rice sheath blight severity and increased rice yields in 2012 and 2013 (Table 4.1). The increase in azoxystrobin doses from 0.08 to 0.16 kg a.i./ha did not contribute to an additional disease reduction or yield increase in 2012 and 2013 (Table 4.4). There were

no significant interactions between any treatments (cover crop, cultivar and fungicide application) consistently in the time evaluated (Table 4.1).

Table 4.1. Analysis of variance (*P* value) for the effects of *Brassica juncea* cover crop, rice cultivar, fungicide application and their interactions on rice sheath blight severity and grain yield in 2011, 2012 and 2013.

		2011[z]		2012		2013	
Source	**DF**	**Disease severity**	**Yield**	**Disease severity**	**Yield**	**Disease severity**	**Yield**
Brassica cover crop (BC)	1	0.0305	0.119	0.0506	0.1121	0.0126	0.0245
Cultivar (CV)	1	-	-	0.0127	0.0014	<0.0001	0.0080
BC x CV	1	-	-	0.6925	0.9947	0.8588	0.0118
Fungicide (F)	2	<0.001	0.3021	<0.0001	0.0009	<0.0001	0.0025
BC x F	2	0.1711	0.7266	0.5218	0.8448	0.2878	0.5985
CV x F	2	-	-	0.5208	0.3137	0.0042	0.8360
BC x CV x F	2	-	-	0.0024	0.9275	0.9857	0.7436

[z] Only one cultivar was evaluated in 2011.

Table 4.2. Effect of *Brassica juncea* cover crop on rice sheath blight severity and grain yield in 2011, 2012 and 2013.

Treatment	Disease severity[y]			Yield (kg/ha)		
	2011	**2012**	**2013**	**2011**	**2012**	**2013**
Fallow	2.3a[z]	5.8a	4.4a	5,372a	7,659a	11,269b
Brassica cover crop	1.4b	3.8b	3.3b	6,360a	7,940a	11,881a

[y]Sheath blight was rated 1 wk before harvest on a 0 to 9 scale where 0 = no disease and 9 = plants dead and collapsed.

[z] Means in the same column with the same letter are not significantly different at $P = 0.05$.

Table 4.3. Effect of rice cultivar on rice sheath blight severity and grain yield in 2012 and 2013.

Cultivar	Disease severity[y]		Yield (kg/ha)	
	2012	**2013**	**2012**	**2013**
Presidio	3.8b[z]	3.2b	8348a	11882a
Cocodrie	5.8a	4.6a	7251b	11267b

[y]Sheath blight was rated 1 week before harvest on a 0 to 9 scale where 0= no disease and 9 = plants dead and collapsed.

[z] Means in the same column with the same letter are not significantly different at $P = 0.05$.

Table 4.4. Effect of azoxystrobin application on rice sheath blight severity and grain yield in 2011, 2012 and 2013.

Application rate (kg a.i. /ha)	Disease severity[y]			Yield (kg/ha)		
	2011[b]	2012	2013	2011	2012	2013
0	3.7a	6.0a	5.9a	5403a	7355a	11170a
0.08	1.6b	4.6b	3.1b	5919a	7770b	11595b
0.16 (label rate)	1.2c	3.7b	2.6b	5919a	8273b	11959b

[y]Sheath blight was rated 1 wk before harvest on a 0 to 9 scale where 0 = no disease and 9 = plants dead and collapsed.

[z] Means in the same column with the same letter are not significantly different at $P = 0.05$.

DISCUSSION

This is the first report of the field efficacy of *B. juncea* cover crop showing the reduction of the severity of sheath blight in three consecutive years in the field. Consequently, these field evaluations suggest that when *B. juncea* cover crop is used in combination with a fungicide, amount of fungicide used can be reduced. This finding is extended from our previous laboratory studies showing that isothiocyanates released from hydrolysis of glucosinolates in *B. juncea* plant tissue reduced the growth of mycelium of *R. solani* (Handiseni et al. 2013).

Our findings on the suppression of rice sheath blight by *B. juncea* cover cropping are in agreement with the previous reports that *B. juncea* cover cropping reduced various soilborne diseases (Lazzeri et al. 2003; Little et al. 2004; Hartz et al. 2005; Larkin and Griffin 2007; Motisi et al. 2009; Snapp et al. 2007; Njoroge et al. 2008). For example, strawberry (*Fragaria* × *ananassa* Duch.) plots amended with *B. juncea* resulted in the reduction of *R. solani* inoculum by 40 to 56% and reduced incidence and severity of root disease symptoms (Larkin and Griffin 2007). Similarly, in the current field study, *B. juncea* cover crop reduced rice sheath blight severity by 10 to 39%.

B. juncea green manure suppressed *R. solani* more effectively in strawberry compared to non-glucosinolate-containing green manure (Larkin and Griffin 2007). In addition to releasing isothiocynates, incorporation of *B. juncea* plant tissue can benefit crop production by improving overall root health (Snapp et al. 2007). Therefore, sheath blight suppression by *Brassica* plant tissue is speculated to result from not only immediate biocidal effects of isothiocyanates but also other mechanisms of disease

suppression. *R. solani* suppression may be associated with the increase of various beneficial soil microbes as a result of the release of isothiocyanates. More research is needed to elucidate the impact *B. juncea* tissue has on soil microbes, and to understand how the resulting microbial shifts suppress *R. solani*.

Brassica seed meals have been successfully used for pest management in crop production (Mazzola et al. 2007). However, in rice cropping systems, use of *B. juncea* cover crop tends to be more suitable than using *Brassica* seed meals because *B. juncea* cover cropping is more practical for a large scale of application. In addition, *B. juncea* cover crop may reduce adverse effects such as phytotoxicity. *B. juncea* plant tissue incorporated in rice fields contains a relatively lower concentration of glucosinolates than *B. juncea* seed meals. Use of *Brassica* seed meals is frequently associated with phytotoxicity (Handiseni et al. 2012).

Brassica juncea cover cropping enables rice farmers to reduce the use of fungicides for management of sheath blight. In the current study, half of manufacturer's label rate (0.08 a.i./ha) of azoxystrobin achieved the same level of sheath blight suppression as the standard label rate (0.16 kg a.i./ha). Even though *B. juncea* cover cropping did not always result in yield increase, a reduction in sheath blight severity by *B. juncea* cover cropping has the potential to reduce the use of fungicides, increase profit margins and promote environmental stewardship.

CONCLUSIONS

The current study demonstrates that *B. juncea* cover cropping can be added to the rice disease management practices including host resistance and fungicide. With further field efficacy validations, the use of *B. juncea* cover crop can enhance the efficacy of the current integrated pest management program for sustainable control of sheath blight in rice.

CHAPTER V

CONCLUSIONS

B. juncea species outperformed other species evaluated probably because they contain high levels of allyl glucosinolate type that results in AITCs release upon hydrolysis. The mycelial inhibition resulting from *B. juncea* soil amendments evaluated in this study seem to be consistently the same pattern when Texas and Arkansas but was different when Mississippi soils were used. This finding confirms the major role of soil properties in biofumigation. As the amount of *B. juncea* plant tissue amended into soil increases, AITC emission increases and consequently antifungal activity becomes stronger. The lower AITC levels observed in pasteurized soils than in natural soil can be attributed to reduced microbial or enzymatic activities by soil pasteurization. This study suggests that even though total glucosinolate content in *B. juncea* amendments is a critical factor in biofumigation efficacy, activities of soil microbes also plays an important role in glucosinolate hydrolysis. The role of soil microbes should not be overlooked in screening for brassicaceous plants for biofumigation. *Brassica juncea* cv. Brand 199 shows great potential as a candidate for biofumigation to manage sheath blight in rice.

B. juncea soil amendment can reduce the viability and aggressiveness of mycelium and sclerotia and affect the formation of sclerotia of *R. solani* AG1-1A. Amount of *B. juncea* plant tissue used for soil amendment was positively correlated to the reductions in viability and aggressiveness of *R. solani* AG1-1A. This information will

be helpful to develop and facilitate the use of brassicaceous biofumigation for management of sheath blight in rice. The two propagule types, mycelium and sclerotia, of *R. solani* AG1-1A responded differently to *B. juncea* in viability and aggressiveness. This implies that proper use of *B. juncea* soil amendment before rice planting has potential to reduce the amount of effective primary inoculum. Since sheath blight is a monocyclic disease, *B. juncea* biofumigation used to reduce primary inoculum in the forms of sclerotia or mycelium would be much effective in reducing sheath blight development in a rice-cropping season.

To our knowledge this is the first report of the field efficacy of *B. juncea* cover crop showing the reduction of the severity of sheath blight in the field. Consequently, these field evaluations suggest that when *B. juncea* cover crop is used in combination with a fungicide, amount of fungicide used can be reduced. *Brassica juncea* cover cropping enables rice farmers to reduce the use of fungicides for management of sheath blight. In the current study, half of manufacturer's label rate (0.08 a.i./ha) of azoxystrobin achieved the same level of sheath blight suppression as the standard label rate (0.16 kg a.i./ha). Even though *B. juncea* cover cropping did not always result in yield increase, a reduction in sheath blight severity by *B. juncea* cover cropping has the potential to reduce the use of fungicides, increase profit margins and promote environmental stewardship.

Overall, our findings demonstrates that *B. juncea* cover cropping can be integrated in rice disease management practices with host resistance and fungicide to effectively reduce sheath blight.

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