Sclerotinia sclerotiorum: Yield Loss of _Brassica carinata_ in the Southeastern United States and Effect of Fungicides and Light Wavelength.

By
Eric Gregory Melby

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Approved by

Kira Bowen, Chair, Professor of Plant Pathology & Interim Department Head of Crop, Soil, and Environment Sciences

Amanda Strayer-Scherer, Assistant Professor of Plant Pathology & Extension Specialist

Edward J. Sikora, Professor of Plant Pathology & Extension Specialist

Abstract

Sclerotinia sclerotiorum is a major pathogen of over 400 plant species around the world and is considered to be the biggest threat to the production of *Brassica carinata* in the southeastern United States. *Brassica carinata* is an oil seed crop commonly grown during the summer in parts of Canada and the midwestern United States. It is currently being investigated for suitability as a winter cash crop in the southeastern United States. The present work addressed several topics concerning *S. sclerotiorum* isolates from the southeastern United States. A study on the effect of light wavelength on carpogenic germination was inconclusive. A study on the effect of light wavelength on biomass accumulation indicated that light wavelength was inconclusive but indicated that photoperiod may be important. In the study on *B. carinata* production, no treatment had a significant effect on yield or disease incidence/severity. It should be noted that environmental conditions in the 2021/2022 growing season were severe and likely skewed the results of this study. Finally, several commercially available fungicides were tested for *in vitro* efficacy against *S. sclerotiorum.* Fungicides included Caramba (metconazole), Sphaerex (metconazole + prothioconazole), Endura (boscalid), and Quadris (azoxystrobin). Quadris' inhibition was significantly lower than the inhibition of all other tested fungicides.

Table of Contents

Abstract 2
List of Tables 5
List of Figures 6
1. Literature Review 7
  Background on Host and Disease 7
  Yield Loss 9
  Management 10
  Effects Of Light Wavelength and Intensity on Fungal Growth 11
  Literature cited 14
2. Effects of Light Wavelength on Carpogenic Germination of *Sclerotinia sclerotiorum* 18
  Abstract 18
  Introduction 18
  Materials and Methods 19
  Results 21
  Discussion 21
  Literature Cited 23
3. Biomass Accumulation of *Sclerotinia Sclerotiorum* in Different Light Wavelengths ... 24
  Abstract 24
  Introduction 24
  Materials and Methods 25
  Wavelength Experiment 25
  Results 28
  Discussion 28
  Literature cited 29

Figures 30
4. The Effects of Fungicide Timing, Cultivar, and Sclerotinia Stem Rot on Yield of *Brassica carinata* 32
Abstract 32
Introduction 32
Materials and methods 34
Results 35
Discussion 36
Literature Cited 38
Tables and Figures 39
V. In vitro Fungicide Efficacy Against Sclerotinia sclerotiorum 47
Abstract 47
Introduction 47
Materials and Methods 48
Results 50
Discussion 51
Literature cited 54
Tables and Figures 55

List of Tables

Table 2.1 Description of disease rating for S. sclerotinia on B. carinata. 39

Table 2.2 Cultivars, fungicide timings, and interactions of the two that were significantly different in yield of B. carinata in the 2021/2022 production season. 46

Table 5.1 Additional information on fungicides tested for in vitro against S. sclerotiorum. 55

List of Figures

Figure 3.1. Iteration 1 of biomass accumulation for each isolate in each color (wavelength) treatment. 30

Figure 3.2. Iteration 2 of biomass accumulation for each isolate in each color (wavelength) treatment. 31

Figure 4.1. *S. sclerotiorum* DSI on *B. carinata* for each fungicide timing in 2020-2021 season. 40

Figure 4.2. *B. carinata* yield for each fungicide timing in the 2020-2021 season. 41

Figure 4.3. 2020-2021 *B. carinata* yield plotted against DSI of *S. sclerotiorum*, separated by cultivar. Fit statistics shown. 42

Figure 4.4. DSI of *S. sclertoriorum* on *B. carinata* for each fungicide timing in the 2021-2022 season. 43

Figure 4.5. 2022 *B. carinata* yield for each fungicide timing in the 2021-2022 season. 44

Figure 4.6. 2022 *B. carinata* yield plotted against disease severity index, separated by cultivar. Fit statistics also shown. 45

Figure 5.1. Mean inhibition for each fungicide and isolate for the first trial, for each concentration. Treatments with the same letter in each concentration are not significantly different from each other. 56

Figure 5.2. Mean inhibition for each fungicide and isolate for the second trial, for each concentration. Treatments with the same letter in each concentration are not significantly different from each other. 57

1. Literature Review

Background on Host and Disease

Brassica carinata is a flowering mustard in the *Brassicaceae* family. It has many common names but, in most research papers, it is usually referred to as Ethiopian cabbage. *Brassica carinata* is an amphidiploid hybrid of *B. nigra* and *B. oleracea* that was cultivated in Ethiopia starting around 3000 BC (Alemayehu and Becker 2002). Until relatively recently, the leaves of *B. carinata* were used as food and the seeds were crushed for food oil. However, due to the high erucic acid content, it was banned as a food crop in the US in the 1950s until low erucic acid cultivars were introduced in the 1970s (Pua and Douglas 2004). The most prevalent adverse effect of erucic acid consumption is its reduction of heart muscle functionality in mammals (Vetter et al. 2020).

Ironically, the erucic acid that was once bred out of *B. carinata* is now its most desirable trait due to it being a very long chain fatty acid (22 carbon molecules in length) (Marillia et al. 2014). Very long chain fatty acids are not typically present in other widely available plant-based oils such as soybean oil or canola oil (Marillia et al. 2014). Very long chain fatty acids are useful in several different industrial and manufacturing applications including lubricants, surfactants, and biodiesel (McVetty et al. 2016).

Sclerotinia sclerotiorum is the causal agent of Sclerotinia stem rot (SSR); a major disease of *B. carinata* as well as *Glycine max* (soybean), *Brassica napus* (canola), *Pisum sativum* (field pea) (Boland and Hall 1994) and over 400 other plant species around the world (Heffer Link and Johnson 2007). Conditions that favor development of SSR include cool, humid, and wet weather (Mueller 2014) . Typical symptoms of *S.*

sclerotiorum infection include wilted leaves and bleached stems; signs include fluffy white mycelium and sclerotia formed on and inside dead plant tissue (Heffer Link and Johnson 2007). *Sclerotinia sclerotiorum* typically infects its host via ascospores. Since ascospores do not have the ability to infect a healthy plant directly, they generally colonize weaker tissue such as senescent flower petals and leaves where they grow saprophytically and gain energy until they can infect healthy plant tissue (Heffer Link and Johnson 2007). Plant tissue is infected via hyphal mechanical pressure through the cuticle or through openings such as wounds or stomata (Sharma et al. 2015). Cell death occurs ahead of advancing hyphae due to fungal production of oxalic acid, which lowers the pH of the environment making conditions more favorable for enzymatic degradation of the plant (Sharma et. al 2015). Once there is little to no nutrition remaining for the pathogen, sclerotia are formed on and in dead plant tissue. Sclerotia overwinter and germinate the following spring, restarting the infection cycle (Agrios 2005).

Sclerotia, while not unique to *S. sclerotiorum*, are a distinguishing feature of this fungus and play a vital role in the life cycle of this genus. Herein, sclerotia are described as they pertain to *S. sclerotiorum.* Sclerotia are nutrient rich resting structures composed of compacted hyphae (Willetts and Bullock 1992). Sclerotia form in and on dead plant tissue when *S. sclerotiorum* has depleted its host of nutrients. Sclerotia are often dislodged into the soil where they are capable of remaining dormant for over three years until conditions are favorable for germination and reproduction (Agrios 2005). *Sclerotinia sclerotiorum* germinates either by carpogenic or myceliogenic germination. Prior to germination sclerotia sometimes must be conditioned for a period in cool temperatures depending on the geographic origin of the *S. sclerotiorum* isolate (Huang and Kozub

1991). Carpogenic germination refers to the production of apothecia on the surface of the sclerotia which, in turn, release ascospores. Myceliogenic germination refers to the direct spread of mycelium from the sclerotia. While all *Sclerotinia* species can germinate by both means, each species has a preferred method. *Sclerotinia sclerotiorum* epidemics driven primarily by carpogenic germination (Heffer Link and Johnson 2007).

Yield Loss

To date, there is little published on the effects of SSR on yield loss of *B. carinata* in the southeastern United States. Therefore, this section relies on yield loss studies, both in other regions as well studies on yield loss of other crops commonly afflicted by SSR. In 2009, SSR ranked as the second most destructive disease of soybeans causing a loss of approximately 560 million dollars in the United States (Peltier 2012). The average annual loss of *Brassica napus* in Australia due to *S. sclerotiorum* is approximately 10.1 million dollars per year (Murray and Brennan 2012).

Del Río et al. (2007) evaluated the impact of *S. sclerotiorum* on canola yield in North Dakota and Minnesota. Research results demonstrated that for every percent increase in SSR incidence, yield potential was reduced by 0.5% (12.75 kg/ha), which at the time of publication, meant 17% incidence would cause the same economic impact as a fungicide treatment. Similar results were observed in Alberta Canada twenty years earlier by Morral et al. (1984) which meant that little has been done to mitigate the impact SSR over the years. However, this also means that virulence in *S. sclerotiorum* has not increased. However, the geographic range of *S. sclerotiorum* has expanded overtime as it was reported on *B. carinata* in Florida for the first time in 2012 (Young et al. 2012).

Management

Host resistance is considered the only long-term, cost-effective means of reducing incidence and severity of SSR in rapeseed oil crops (Barbetti et al. 2013). However, identifying resistant *B. carinata* lines is difficult due to environmental factors that affect SSR virulence (Kithul-Pelage et al. 2013, Uloth et al. 2015). Furthermore, the effectiveness of cultivar selection can be influenced by inoculum potential (Mueller et al. 2002). A study revealed that silencing a gene responsible for production of reactive oxygen species (ROS) inhibited development of SSR in the *G. max*. This is because the oxalic acid produced by *S. sclerotiorum* induces program cell death, which is reliant on ROS (in essence, *S. sclerotiorum* relies on plant participation to be pathogenic) (Ranjan et al. 2017). The effect of reducing ROS to delay programmed cell death and inhibit *S. sclerotiorum* spread in the host was also confirmed in *B. napus* (Wen et al. 2013). As of 2019, efforts to identify commercially suitable and disease-resistant varieties of *B. carinata* are ongoing (Seepaul 2019). Therefore, control via fungicides is extremely important for managing SSR.

While multiple fungicides have been identified as effective in preventing the onset of SSR (Gorman 2020, Mueller et al. 2002), it is important to identify additional effective fungicides and use them either in rotation or in combination with fungicides with a different mode of action. This ensures that isolates that become resistant to one fungicide succumb to the alternative fungicide rather than gaining resistant genes in the population (Bradley et al. 2006, Brent and Hollman 2007). Most fungicides registered for the control of SSR are either demethylation-inhibiting (DMI; FRAC group 3), succinate dehydrogenase inhibiting (SDHI; FRAC group 7), or quinone outside inhibiting (QOI;

FRAC group 11) These fungicides have a single site mode of action which makes them more susceptible to resistance developing in the pathogen, because a single gene in the pathogen controls resistance. Conversely, several genes of a pathogen would need to change to resist a multisite fungicide. Not all chemistries within these groups are efficacious. Gorman (2020) reported that flutriafol (group 3) was not effective at inhibiting growth of *S. sclerotinia in vitro,* while Mueller et al. (2002) reported that tebuconazole (group 3) was effective; Liu et al. (2021) reported metconazole (group 3) was highly effective against *S. sclerotiorum*. This indicates the need for further research on effective chemistries both in, and outside of the groups listed above. The mitigation of resistance is especially important for group 11, which is listed as being at high risk for resistance development. Group 7 is listed as a medium-high risk and group 3 is listed a medium risk. This indicates the need for further research on effective chemistries both in and outside of the groups listed above. In addition to preventing fungicide resistance, identifying and registering more fungicides as effective controls of SSR could contribute to driving down the price of fungicides which increases profit margins for growers (Bradley et al. 2006).

Effects Of Light Wavelength and Intensity on Fungal Growth

Different wavelengths and intensities of light affect several aspects of some fungal species' physiology including biomass production (Poyedinok et al. 2008, Zapata et al. 2009), secondary metabolite production (Haggblom and Unestam 1979, Pruss 2014 et al.) the relative size of the constituent parts (such as the pileus and stipe) (Jang et al. 2013), their molecular properties (Faneli et al. 2012, Poyedinok et al. 2008), and it can also influence reproductive behavior (Blumenstein et al. 2005). The results of these

effects are of interest to several different fields such as fungal cultivation and plant pathology studies. The majority of studies done on the effects of light on fungi focused on the reaction of an individual species. Often the study is focused on a particular reaction such as biomass production or changes in molecule production. Furthermore, often the molecular reason for the changes observed are unknown or not discussed in the literature. Thus, it is not prudent to suppose a particular light treatment will significantly affect all fungi in the same way. However, for many purposes (especially those of cultivation) the application of certain light conditions for certain outcomes is still viable despite the lack of understanding of the specific mechanisms (Poyedinok et al. 2008).

The effects of light color, light intensity, and light duration on the carpogenic germination of sclerotia of the genus *Sclerotinia* have been previously reported. Bedi (1962) floated sclerotia of *S. sclerotiorum* on sterile water in Erlenmeyer flasks that were each covered with different colored cellophane bags and placed in front of a window. Results indicated that light is not necessary for stipe-only germination but is necessary for apothecia production. Bedi (1962) concluded that white light was best for apothecia production while red and green lights are poor. The results for red and blue light germination are interesting compared to proceeding research; red light resulted in low carpogenic germination rates while blue light resulted in high stipe germination with no apothecia. One issue with this study is that cellophane has a broad transmission range, meaning that while the cellophane may be green in color, the wavelengths allowed to pass may be outside the 495-570 nanometer range (Honda and Yunoki 1975). In addition, there was a lack of control over light intensity and the measurement of light

intensity was imprecise. Singh and Singh (1987) conducted a similar experiment using glass filters, which were much more effective in filtering specific wavelengths, the light source was an incandescent bulb, and light intensity was accurately measured with a solarmeter. In contrast to findings by Bedi (1962), Singh and Singh (1987) found that blue light stimulated the highest production of apothecia and did so more quickly than other light colors. Differences in these results could be due to the previously mentioned issues with wavelength transmission of cellophane. It is also possible that the light source and photoperiod impacted results since the sun and an incandescent bulb transmit different wavelengths of light. Furthermore, Singh and Singh (1987) had to take precautions to prevent the heat of the light source from interfering with the experiment. Contrary to Bedi (1962), Thanning and Nilson (2000) determined that apothecia could be formed in blue light. However, the apothecia were not fully expanded relative to apothecia produced with other wavelength treatments indicating that blue light may not be the most efficient wavelength for apothecia generation in contrast to the findings of Singh and Singh (1987).

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2. Effects of Light Wavelength on Carpogenic Germination of *Sclerotinia sclerotiorum*

Abstract

Sclerotinia sclerotiorum is a major pathogen of over 400 plant species around the world and is considered to be the biggest threat to the production of *Brassica carinata* in the southeastern United States. The present study examined the effects of different light wavelengths on carpogenic germination of *S. sclerotiorum.* Tested light treatments included blue, green, red, white, yellow, and no light. After the first iteration of this experiment yielded no germinations, the experiment was repeated with a different conditioning procedure and photoperiod, which also yielded no germinations. It is suspected that the surface sterilization of the sclerotia prior to testing may have been gratuitous and may have rendered them unviable. Furthermore, the second trial conditioning procedure was based on a previously published report which did not describe moisture conditions. Use of inappropriate moisture could have contributed to lack of germination.

Introduction

Many fungi are photosensitive and will often have either enhanced or inhibited physiological function depending on the fungus and wavelength/intensity of light. Several studies have been done to determine the impact of light wavelength on apothecia germination of the pathogenic fungi *Sclerotinia sclerotiorum*. Bedi (1962) determined that light is not required for stipe-only germination but it is necessary for apothecia germination. Bedi (1962) also concluded that white light was best for apothecia production. Red, orange, yellow, and green lights had mixed results, but they all reduced apothecia production. Darkness and blue light were completely inhibitory to

apothecia formation. Singh and Singh (1987) confirmed that light was not necessary for apothecia formation. However, they found that blue light stimulated the greatest number of apothecia germination and did so more quickly than other light colors, including white light. More recently Thaning and Nilsson(2000) determined that apothecia could be formed in blue light but were not fully expanded relative to apothecia from other wavelength treatments in their study. This indicated that blue light may not be the most efficient wavelength for apothecia generation in contrast to the findings of Singh and Singh (1987).

The present research sought to clarify what wavelength of light produces the most apothecia by using LED bulbs emitting different wavelengths of light rather than using light filters has been done in prior work with *S. sclerotiorum*.

Materials and Methods

Fungal Isolates and Storage

The *S. sclerotiorum* isolates were named according to their source location: Blairesville, GA (BR), Brewton, AL (BT), Tifton, GA (TI), and Quincy, FL (QC). Isolates sourced by a former lab member who collected sclerotia from *B. carinata* fields in each location. These sclerotia were stored at room temperature for an unknown period of time in coin envelopes at room temperature (19°-21°C) prior to use in this experiment. It is likely that these sclerotia did not originate from the field but were generated from original sclerotia according to Pethybridge et al. (2015) by the former lab member.

Wavelength Experiment

Sclerotia from each isolate were surfaced sterilized according to Rollings (2007) and incubated on PDA at room temperature (19°-21°C) for seven days. The resulting

colonies were used to produce sclerotia for this experiment according to Pethybridge et al. (2015). Briefly, 25g of wheat grain was placed in a 500ml Erlenmeyer flasks with 50 ml of water, which were then sealed with aluminum foil and sterilized. The sterilized wheat was inoculated with four mycelial plugs 7mm mycelial plugs and incubated at room temperature (19°-21°C) until sufficient sclerotia were observed. Sclerotia were collected by drying the entire contents of the flasks on paper towels in a biological safety hood for 24 hours. Sclerotia were then separated from the wheat, sorted by size, and stored in coin envelopes at room temperature (19°-21°C) until needed.

Prior to conditioning, sclerotia were surface sterilized according to Rollins (2007). Sclerotia were conditioned according to Sun and Yang (2000) in the first iteration of this experiment by placing sclerotia on sterile paper towels, in sterile petri dishes, and moistening with sterile DI water. Plates were then sealed with parafilm and refrigerated at 4°C for 60 days. In the second iteration of this experiment sclerotia were conditioned according to Gorman (2020) by burying the sclerotia in sterile sand, then placing them in a growth chamber set to uniform conditions of 18/6°C day/night (12h cycle) for six weeks.

Six cardboard light chambers were constructed to be used as the test environments. Chambers were approximately $35cm^3$, lined with aluminum foil to evenly distribute the light intensity, and illuminated with a single FEIT Electric ® A19 LED filament bulb (blue, green, red, or yellow) fixed in the center of the lid. All lights were set to $10\mu mol/m^2/s$ (measured with a Hydrofarm Parmeter from the bottom of the chambers) to equalize intensity of the bulbs.

For each isolate, five sclerotia were placed on moistened, sterile sand in a petri dish. Two replicates of each dish were tested in each light treatment. For the first iteration of the experiment light exposure was constant and the temperature was approximately 23°C. The second iteration of this experiment was also conducted at approximately 23°C but the lighting was on an 18/6 hour light dark cycle. In both iterations, sand was remoistened every two-three days with DI water.

Results

Neither iteration produced apothecia on any sclerotia, in any light condition (data not shown.

Discussion

After the first experiment yielded no germination for any treatment, the procedure for second iteration was altered based on a similar study done on the effects on light intensity on carpogenic germination of *S. sclerotiorum*, (Gorman 2020). Since the second iteration also yielded no germination under any treatments it is supposed that the complete lack of results was due to the surface sterilization process. For the second trial, the conditioning procedure followed did not describe moisture conditions. Use of inappropriate moisture could have also contributed to lack of germination Dillard et al. (1995).

A future iteration of this experiment would omit the surface sterilization process, provided the sclerotia used were generated in a sterile environment as was done in the present study. Additionally, future work should be done on a larger scale, with several different conditioning methods tested simultaneously. Not only would this increase the

likelihood of germination, but it would provide insight on conditioning preferences of southeastern US *S. sclerotiorum* isolates.

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3. Biomass Accumulation of *Sclerotinia Sclerotiorum* in Different Light Wavelengths

Abstract

Sclerotinia sclerotiorum is a major pathogen of over 400 plant species around the world and is considered to be the biggest threat to the production of *Brassica carinata* in the southeastern United States. The effect of light wavelength on *S. sclerotiorum* biomass accumulation was investigated. The experiment conducted twice with considerable changes made to the procedure in the second iteration. Neither iteration indicated that wavelength, isolate, or their interaction had a significant effect on biomass accumulation of *S. sclerotinia.* However, in the first iteration, the dark treatment had the lowest biomass accumulation which indicates that light may be an important factor.

Introduction

Different light wavelengths and intensities of light affect several aspects of some fungal species' physiology including biomass production (Poyedinok et al. 2008, Zapata et al. 2009), the relative size of the constituent parts (such as the pileus and stipe) (Jang et al. 2013), their molecular and toxicological properties (Faneli et al. 2012, Poyedinok et al. 2008), and reproductive behavior (Blumenstein et al. 2005).

The present study seeks to identify if certain light wavelengths affect the biomass accumulation of *Sclerotinia sclerotiorum.* There are only a limited number of publications on the describing the impact of light wavelength of *S. sclerotiorum* biomass accumulation. Furthermore, there also is little published about biomass accumulation of any Ascomycete under different light wavelengths.

Sclerotinia sclerotiorum is a pathogen of over 400 plants worldwide (Heffer Link and Johnson 2007). *Sclerotinia sclerotiorum* does not have the ability to infect healthy plant

tissue directly and generally relies on ascospores colonizing senescent flower petals. Following petal infection *S. sclerotiorum* grows saprophytically until it can infect healthy plant tissue (Heffer Link and Johnson 2007). Infection is done via mechanical pressure of the mycelium into the tissue of the plant or via openings such as wounds or stomata (Sharma et al. 2015).Host plant cell death occurs ahead of advancing hyphae due to the fungal production of oxalic acid, which lowers the pH of the environment allowing enzymatic degradation of the plant (Sharma et. al 2015). Once there is little to no nutrition remaining for the pathogen, sclerotia are formed on and in the dead plant tissue. Sclerotia overwinter and germinate in the following spring, restarting the infection cycle (Agrios 2005).

Materials and Methods

Fungal Isolates and Storage

The *S. sclerotiorum* isolates were named according to their source location: Blairesville, GA (BR), Brewton, AL (BT), Tifton, GA (TI), and Quincy, FL (QC). Isolates sourced by a former lab member who collected sclerotia from *B. carinata* fields in each location. These sclerotia were stored at room temperature for an unknown period in coin envelopes prior to use in this experiment. It is likely that these sclerotia did not originate from the field but were generated from original sclerotia according to Pethybridge et al. (2015) by the former lab member. The inoculum for this experiment was generated by incubating these sclerotia on potato dextrose agar plates (PDA) at room temperature (19-21°C) and reculturing subsequent plates with 7 mm mycelial plugs.

Wavelength Experiment

The experiment was done twice with considerable modifications to the procedure in the second iteration.

Iteration 1

A foil lined box (approx. 50 x 40 x 30cm^3) was placed around each arm of a wrist action shaker (Burrell, Carnegie, PA). A hole was then cut into the center of the top of each box to allow the socket of the lamp cord to fit securely. The minimum and maximum light intensity of each bulb color was recorded for each box with a quantum PAR Meter (Hydrofarm, Petaluma, CA). Each bulb was subsequently set to 10 µmol/m^2/s (measured from the bottom center of the box) to account for the low intensity of some bulbs.

Culture broth was prepared by mixing potato dextrose broth (PDB) (HiMedia, West Chester, PA) according to manufacturer's directions. 100ml was transferred to 16 individual 250ml Erlenmeyer flasks, flasks were capped with aluminum foil, and autoclaved. PDB was then inoculated with a seven-day old *S. sclerotiorum* PDA plugs of one of each of four isolates. Flasks were sealed with Parafilm then labeled with isolate ID, light color, and position in the growth chamber. The other eight flasks were treated in the same manner with a different isolate.

The sixteen samples were loaded onto the shaker inside a box four replicates of the two isolates were placed in each box in an alternating manner. The desired light bulb was installed in each chamber and the chambers were then sealed. The shaker ran constantly at 385 oscillations per minute (± 10%) with angle of movement set to 2° for seven days at room temperature (19°-21°C). The above steps for sample preparation and treatment were executed for every combination of light color and isolate (blue, green, red, yellow, white, and dark, with BR, BT, TI, and QC).

After a seven day incubation period the samples were strained through pre-weighed cheese cloth, dried for 24 hours at 55°C (dry-type, Blue m, New Columbia, PA). Following the drying time, the sample weights were recorded.

Iteration 2

Inoculum was changed from seven day-old to four day-old *S. sclerotinia* cultures, with mycelial plugs taken from culture margins. An aluminum foil-lined divider was placed into each chamber such that each chamber had two symmetrical cube sections. A lamp cord was installed to ensure that each chamber had a LED bulb in the center of each cube section to improve the uniformity of the light intensity throughout the chamber. Rather than test multiple isolates simultaneously in the same chamber a single isolate was tested (one isolate, eight replicates). Furthermore, to compensate for the additional time it would take to test each isolate individually, isolate BT was not included nor was the position of each sample recorded.

Data analysis

All statistical analyses were completed using R (R version 4.0.3; 2020-10-10). Data collected from iteration 1 was analyzed using a linear mixed model with light color, isolate, and the interaction of isolate and light as fixed effects; chamber position was a random effect. In contrast, data collected from iteration 2 was analyzed using a regular linear model with light color, isolate, and the interaction of isolate and light as fixed effects. Following this Tukey's test was used to determine which treatments were significantly different from each other. Finally, biomass accumulation of each iteration was plotted on bar plots using the ggplot2 package.

Results

The linear mixed model from the first iteration did not indicate that any isolate, light color, or interaction of the two had a significant effect on biomass accumulation. Analysis of variance on the second iteration showed that the interaction of light and isolate was significant. However, a subsequent Tukey's test did not indicate any significant differences between any treatments. (Mean biomass of each isolate, in each treatment, can be seen in Fig. 3.1 and 3.2)

Discussion

The effects of light wavelength, isolate, and the interaction of the two on the biomass accumulation of southeastern US *S. sclerotiorum* are apparently not significant. However, the biomass accumulation in the first iteration of this experiment is greater than that of the second iteration, indicating that photoperiod may be an important factor in biomass accumulation. Furthermore, in the first iteration, the dark treatment had the lowest biomass accumulation which also indicates that light may be an important factor.

The procedure of this experiment could be refined to obtain more concrete results. For example, the experiments were conducted in the fluctuating ambient temperature of the lab. Thus, it may be important to conduct the experiments in a growth chamber to standardize the incubation temperature. Secondly, the resulting final biomass would be more precisely measured if the cheese cloth and wire strainer were replaced with filter paper and a manifold. Finally, the chamber lining should be replaced with horticultural mylar for better light intensity and distribution.

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Figures

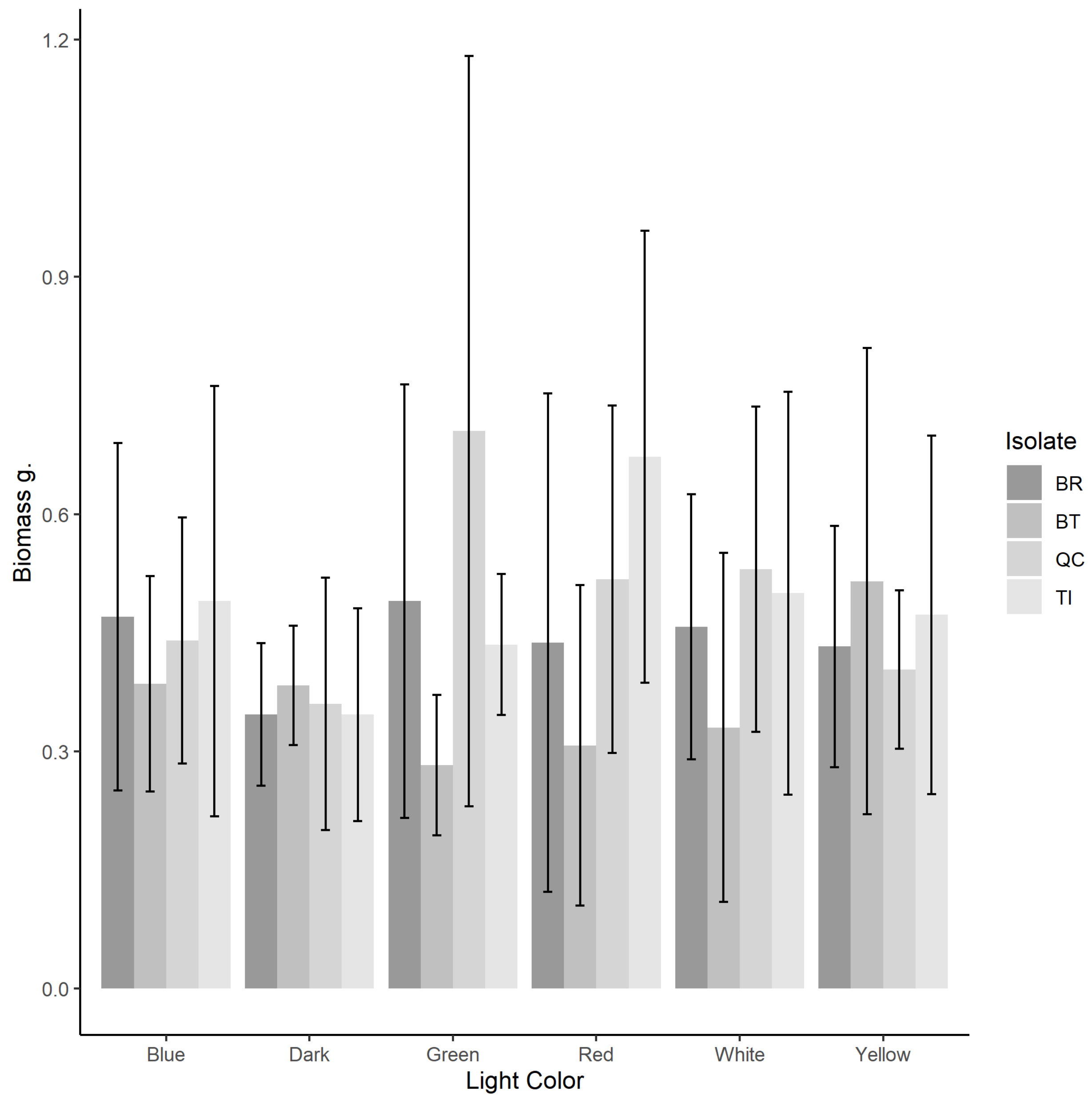

Fig. 3.1. Iteration 1 of biomass accumulation for each isolate in each color (wavelength) treatment.

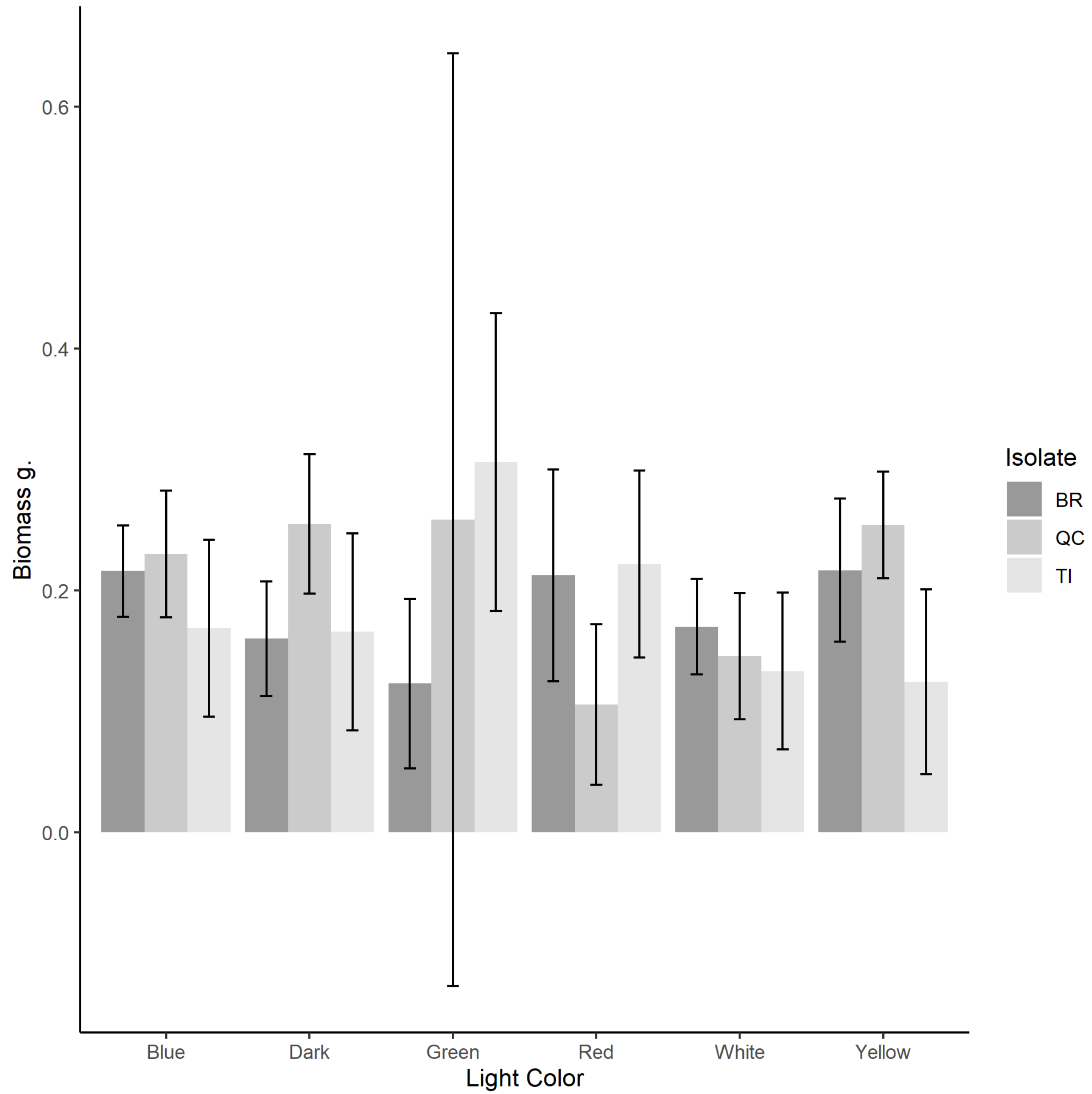

Fig. 3.2. Iteration 2 of biomass accumulation for each isolate in each color (wavelength) treatment.

4. The Effects of Fungicide Timing, Cultivar, and Sclerotinia Stem Rot on Yield of *Brassica carinata*

Abstract

A study was conducted over two growing seasons (2020-2021 and 2021-2022) to determine if cultivar, fungicide timing, or the combination of these factors affected disease severity caused by *S. sclerotiorum* and yield of *B. carinata*. A factorial set of treatments (four cultivars * six fungicide timings) were planted as split plots in each of four blocks. *Brassica carinata* cultivars included AGR044-321E, Avanza 641, DH-040.342, and M-01). Fungicide treatments consisted of a single application of Prosaro at 5.7 fl oz / A (prothioconazole + tebuconazole; Bayer Research Triangle Park, NC) at either GS 30-32, GS 51, GS 60, GS 62-64, GS 67, or non-treated. Main plots were *B. carinata* cultivars and split plots were fungicide timing. While some factors were significantly different according to the model, subsequent Tukey's tests did not indicate that any treatments were significantly different from each other. In the 2020/2021 production season there was positive correlation between DSI and yield in cultivars M-01 (p value = 0.01, R=0.60) and AGR044-321E (p value = 0.01, R=0.59). There was no significant interaction between DSI in the 2021/2022 production season.

Introduction

Brassica carinata is a non-food oilseed crop being investigated for viability as a winter cash crop in the southeastern United States. The oil is rich in very long chain fatty acids that are not typically present in other widely available plant-based oils such as soybean oil or canola oil (Marilla et al. 2014). One of the challenges inherent in growing *B.*

carinata in any region is the pathogen *Sclerotinia sclerotiorum,* causal agent of Sclerotinia stem rot (SSR).

Sclerotinia sclerotiorum is a pathogen of over 400 plants worldwide (Heffer Link and Johnson 2007). *Sclerotinia sclerotiorum* does not have the ability to infect healthy plant tissue directly and generally relies on ascospores colonizing senescent flower petals. Following petal infection *S. sclerotiorum* grows saprophytically until it can infect healthy plant tissue (Heffer Link and Johnson 2007). Infection is done via mechanical pressure of the mycelium into the tissue of the plant or via openings such as wounds or stomata (Sharma et al. 2015). Host plant cell death occurs ahead of advancing hyphae due to the fungal production of oxalic acid, which lowers the pH of the environment allowing enzymatic degradation of the plant (Sharma et. al 2015). Once there is little to no nutrition remaining for the pathogen, sclerotia are formed on and in the dead plant tissue. Sclerotia overwinter and germinate in the following spring, restarting the infection cycle (Agrios 2005).

Host resistance is considered the only long-term, cost-effective means of reducing incidence and severity of SSR in rapeseed oil crops (Barbetti et al. 2013). However, identifying resistant *B. carinata* lines is difficult due to environmental factors that affect SSR virulence (Kithul-Pelage et al. 2013, Uloth et al. 2015). It has been reported that *B. carinata* is moderately tolerant to *S. sclerotiorum* (Yang et al. 2009). Tolerance is defined as the ability of a plant to perform its normal biological functions when infected with a pathogen or pest and is distinct from resistance which is defined as the ability of a plant to limit or eliminate a pathogen or pest colonization (Ney et al. 2012). Therefore, control via fungicides is extremely important for managing SSR.

This study evaluated the effects of four *B. carinata* cultivars and six fungicide application timings on yield. Furthermore, it was of interest to determine if *S. sclerotiorum* incidence and severity correlated with yield loss.

Materials and methods

Field Experiments

For the 2020/2021 production season, a field trial was established in October 2020 at Field Crops Unit, E.V. Smith Research Center in Shorter, AL. A second trial was established in October 2021 at the same location for the 2021/2022 production season. Both trials consisted of a factorial set of treatments (four cultivars X six fungicide timings) planted as split plots in four blocks. Main plots were *B.carinata* cultivars and split plots were fungicide timing. Plots were each 4.1' X 20'. Cultivars included AGR044-321E, Avanza 641, DH-040.342, and M-01. Fungicide timings were either GS 30-32, GS 51, GS 60, GS 62-64, GS 67, or non-treated. Fungicide treatments consisted of a single application of Prosaro (5.7 fl oz / A) (prothioconazole and tebuconazole) (Bayer, Research Triangle PK, NC). In the 2021-2022 field trial, the field borders were seeded with purple-type *B. carinata* (AU052-1) and a border plot was planted every two plots. While the field was still bordered with purple-type *B. carinata* (AU052-1), there were no borders interspersed between plots within the field for the 2021/2022 field trial.

A principal metric for this study was the periodic rating of the disease *Sclerotinia* stem rot. For the 2020/2021 field trial, a 1-m square area was staked early in the season, and stand counts were taken from the entire square meter staked section. In contrast, for the 2021/2022 field trial, stand counts were only taken from the center row of the square meter and disease was rated on this row every other week. Disease incidence and

severity was recorded approximately once a week following the seedling stage. The severity of each incident of disease was rated on a 1-5 scale (Table 4.1). The final disease rating was used to calculate the disease severity index (DSI) for each treatment. DSI was calculated as follows:

100 x Σ(severity rating scale from 0-5 per infected plant)/(5 x stand count)

Data Analysis

Data from each season were analyzed separately.

All data was analyzed in R (R version 4.0.3; 2020-10-10). Two linear mixed models were created, using the lmer package, with cultivar, fungicide timing, and the interaction of the two as fixed effects and range and the interaction of range and cultivar as random effects in the first two. The response variable of the first linear model was DSI and the second model response variable was yield. The emmeans package was used to find significant differences in treatments via Tukey's test.

The ggplot2 package was used to create boxplots showing the distribution of DSI and yield of each treatment by fungicide timing. Finally, DSI was plotted against yield using ggplot2 package and fit statistics were calculated via Pearson correlation using the stat_cor function.

Results

Growing Season 2020/2021

The linear mixed model for determining effect of cultivar, fungicide timing, and their interaction on DSI indicated that the only significantly different treatment was cultivar M-01 (p value = 0.004). However, the subsequent Tukey's test did not indicate that any treatments were significantly different from each other. (Distribution of DSI by fungicide

timing is shown in Fig. 4.1). The linear mixed model for yield indicated there was significant differences in the interaction of cultivar DH-040 and fungicide application timing GS60 (p value = 0.016), as well as the interaction of cultivar Avanza 641 fungicide timing GS67 (p value = 0.045). The subsequent Tukey's test did not indicate that any treatments were significantly different from each other. (Distribution of yield by fungicide timing is shown in Fig. 4.2). Finally, DSI was significantly positively correlated with yield in cultivars M-01 (p value = 0.01, R=0.60) and AGR044-321E (p value = 0.01, R=0.59) (Fig. 4.3).

Growing Season 2021/2022

The linear mixed model for determining effect of cultivar, fungicide timing, and their interaction on DSI did not indicate any significant differences between treatments. (Distribution of DSI by fungicide timing shown in Fig. 4.4). The linear mixed model for yield indicated several significant differences in yield for cultivar, timing, and their interaction (Table 4.2). The subsequent Tukey's test did not indicate that any treatments were significantly different from each other. (Distribution of yield by fungicide timing shown in Fig. 4.5). When DSI was plotted against yield there was no correlation (Fig. 4.6).

Discussion

A previous study on canola observed that, for several different fungicide chemistries, incidence of SSR was inconsistent based on bloom percentage which indicated that the ideal time to apply fungicides is not entirely based on the growth stage of the plant. This study also reported that reducing SSR incidence did not always result in significant yield increase (Bradley et al. 2006). The findings of the 2020/2021 season agree that

fungicide timing should be based on additional factors other than growth stage, such as weather and apothecia development. Furthermore, the results of this season did not indicate that different had a significant effect on yield.

The severity of the environmental conditions that affected the 2021/2022 season cannot be overstated. As a result of weed pressure alone, 16 of 72 plots were removed from the study. Furthermore, density of *B. carinata* was much higher in plots in the northern regions of the field. Since the lower stand count resulted in less canopy cover the microclimate of the plots was much drier, resulting in conditions less favorable to *S. sclerotiorum* infection. The distribution of DSI showed some similarity between seasons despite yield being less than half as much in the 2021/2022 seasons vs the 2020/2021 season (Fig. 4.3 and 4.6), further indicating the effect of the above environment conditions of 2021/2022 season.

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Tables and Figures

Table 4.1. Description of disease rating for S. sclerotinia on *B. carinata.*

Rating	Description
0	No disease
1	Pod infection only
2	Lesion(s) on main or lateral stem such that 25% seed reduction is possible
3	Lesion(s) on stems such that 50% reduction is possible
4	Lesion(s) on stems leading to 75% loss
5	Main stem girdled, no pods or seed

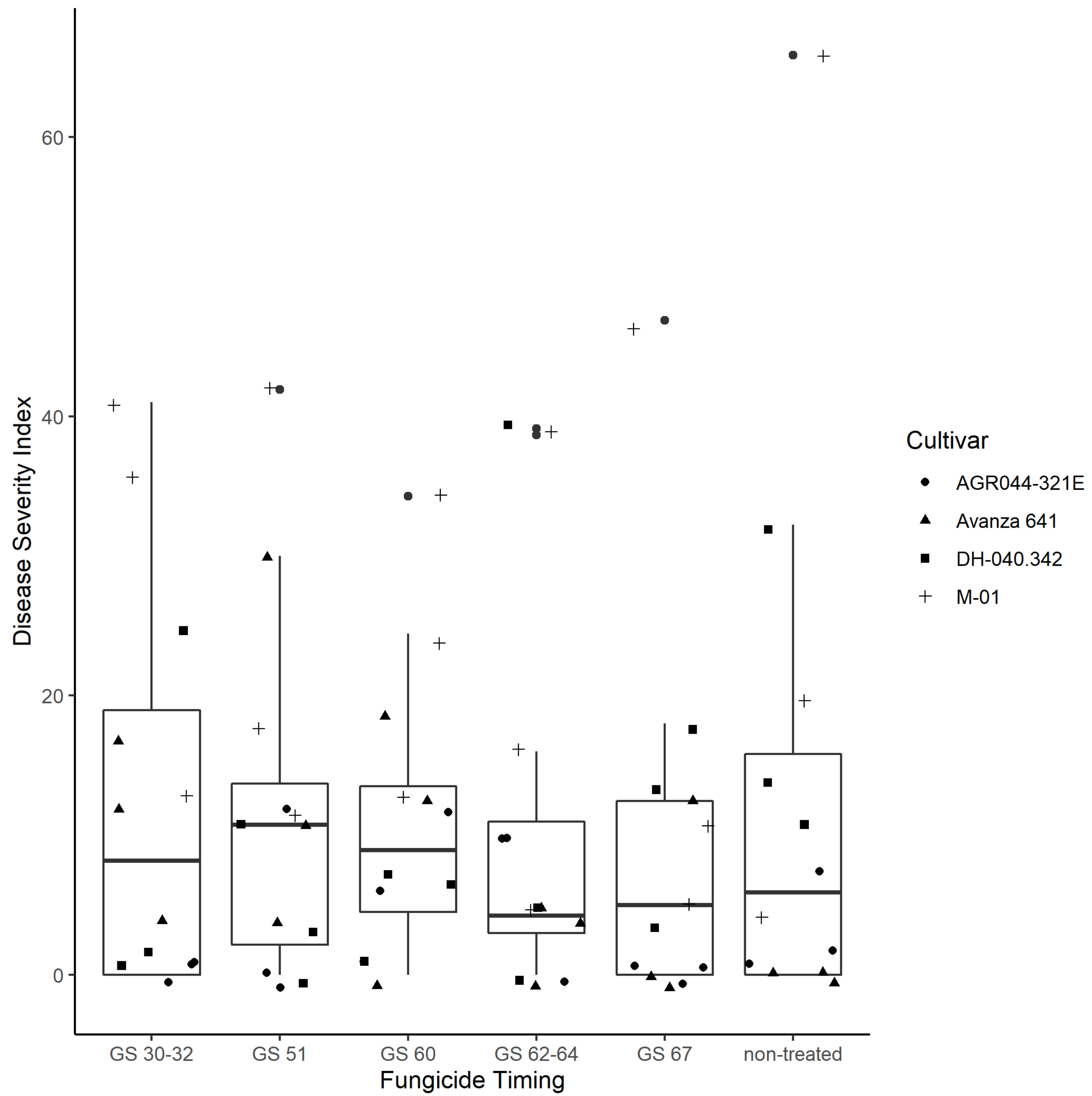

Figure 4.1. *S. sclerotiorum* DSI on *B. carinata* for each fungicide timing in the 2020-2021 season.

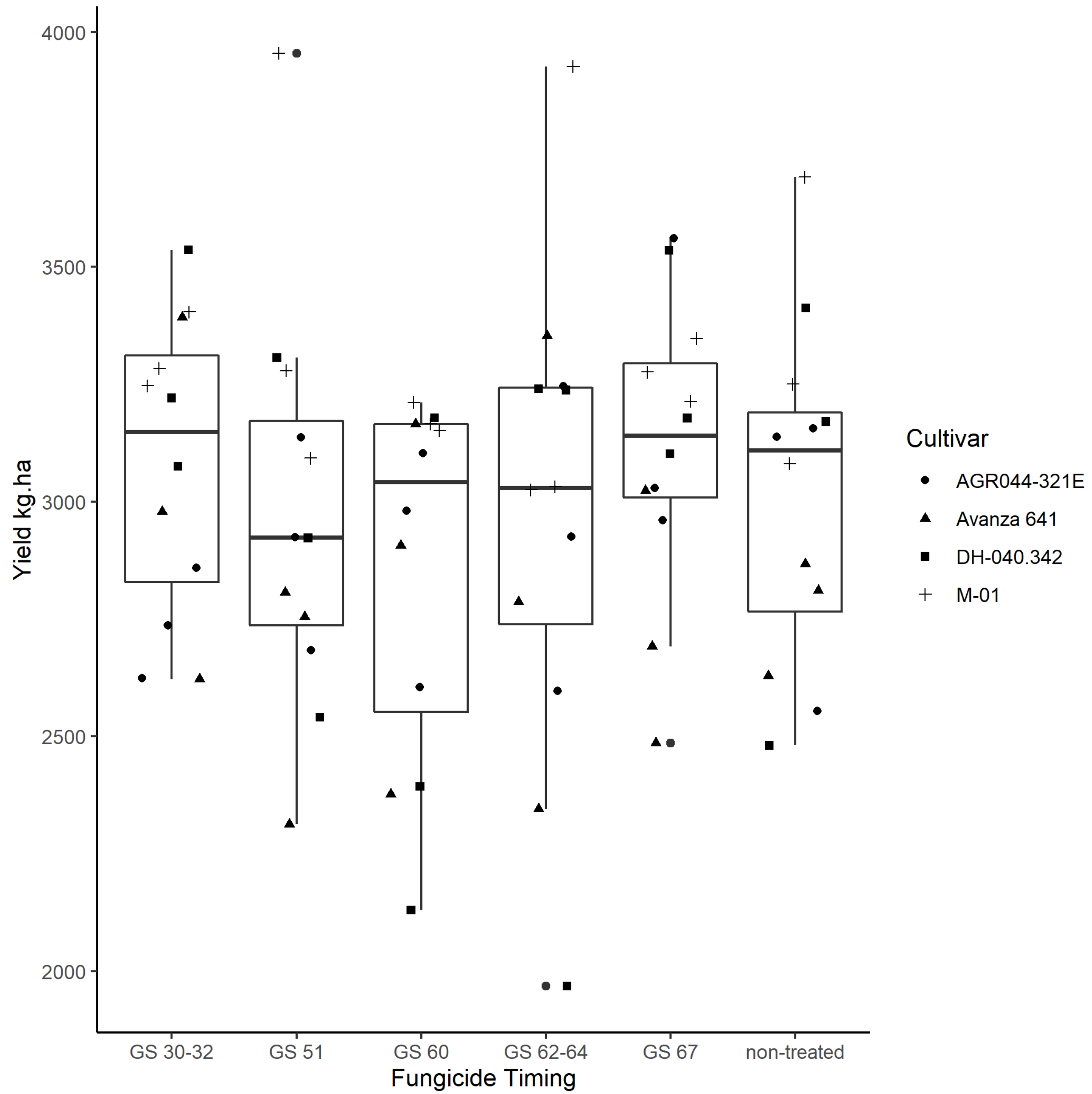

Figure 3.2. *B. carinata* yield for each fungicide timing in the 2020-2021 season.

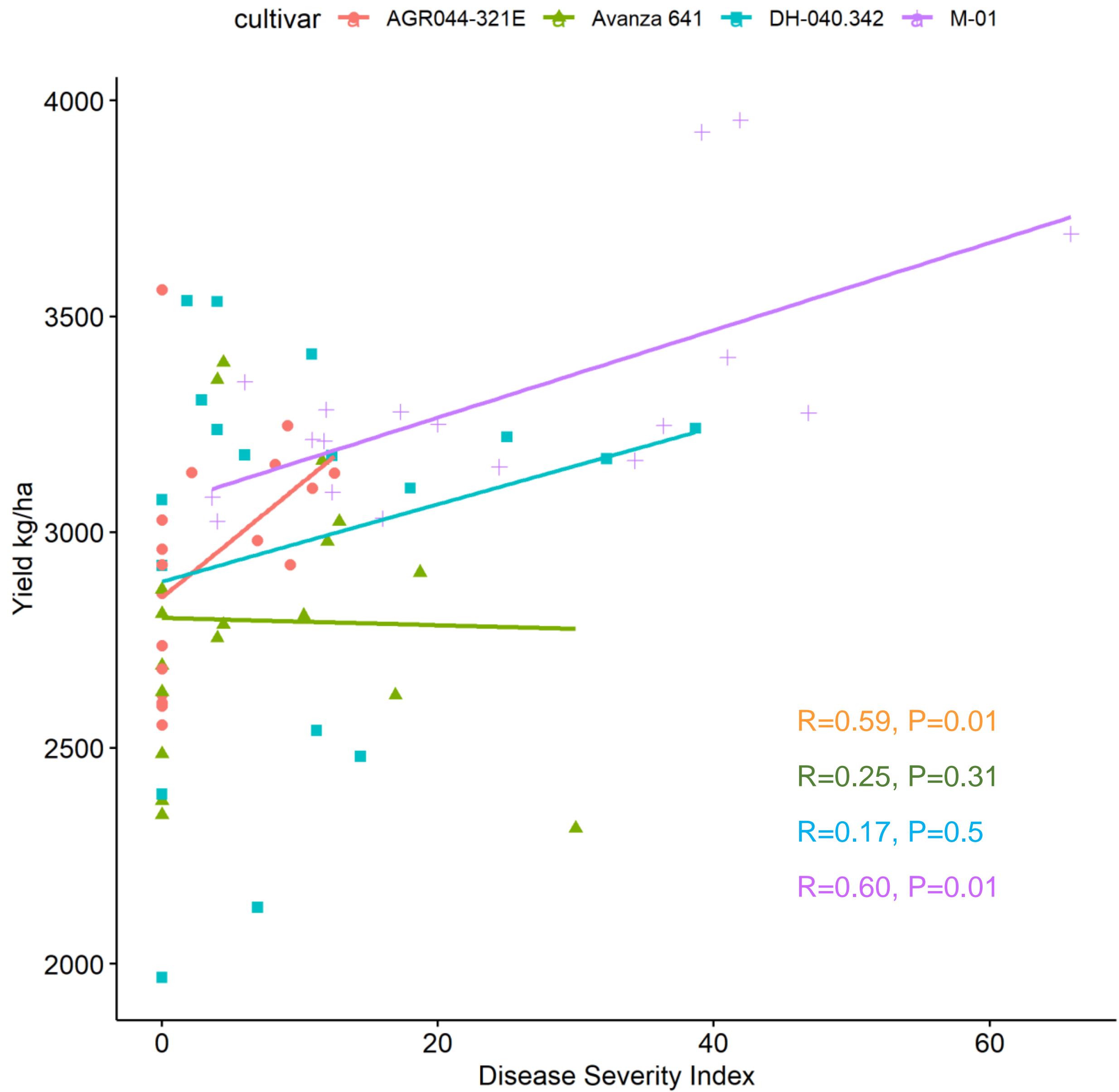

Figure 4.3. 2020-2021 *B. carinata* yield plotted against DSI of *S. sclerotiorum*, separated by cultivar. Fit statistics shown.

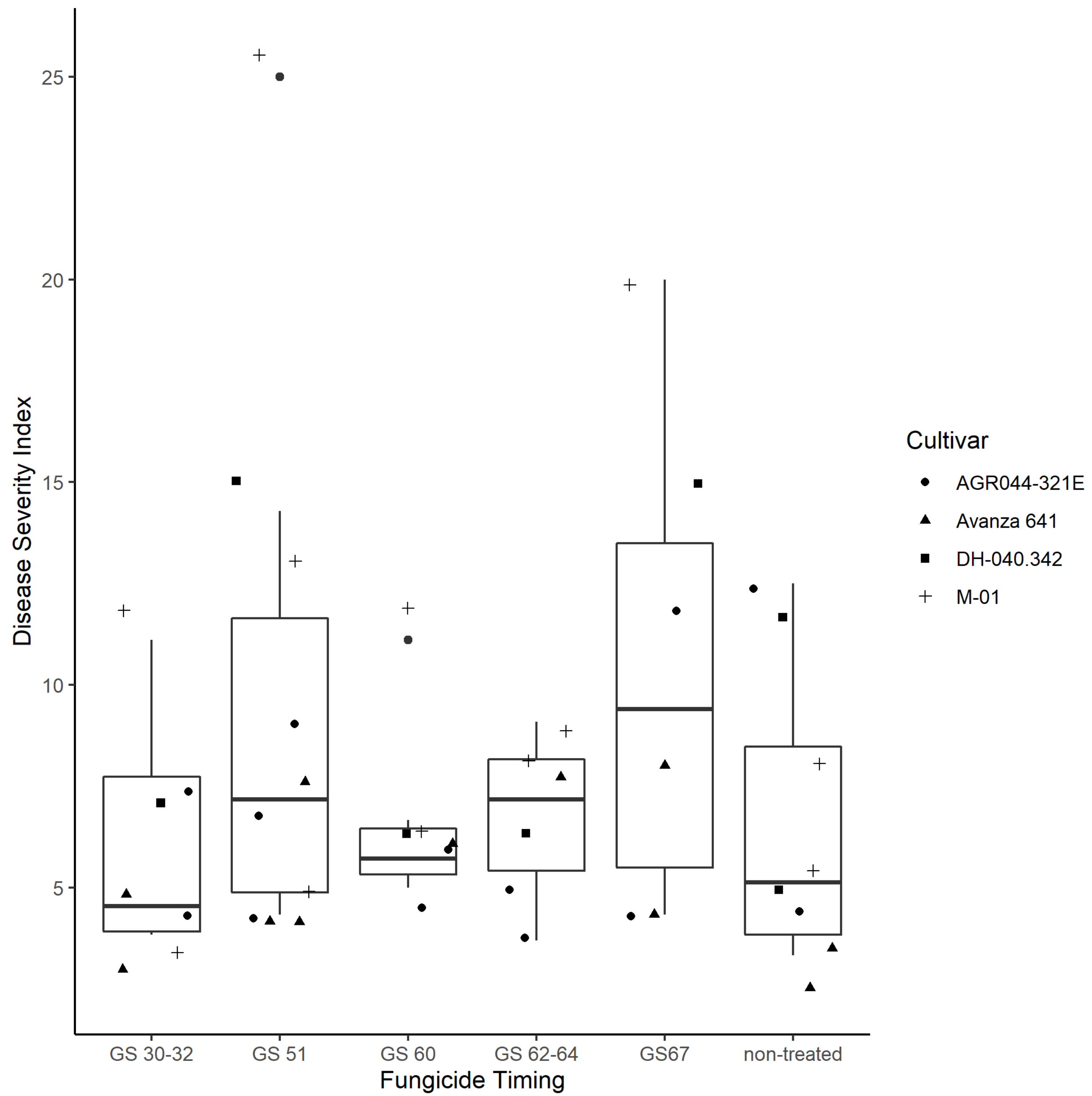

Figure 4.4. DSI of *S. sclertoriorum* on *B. carinata* for each fungicide timing in the 2021-2022 season.

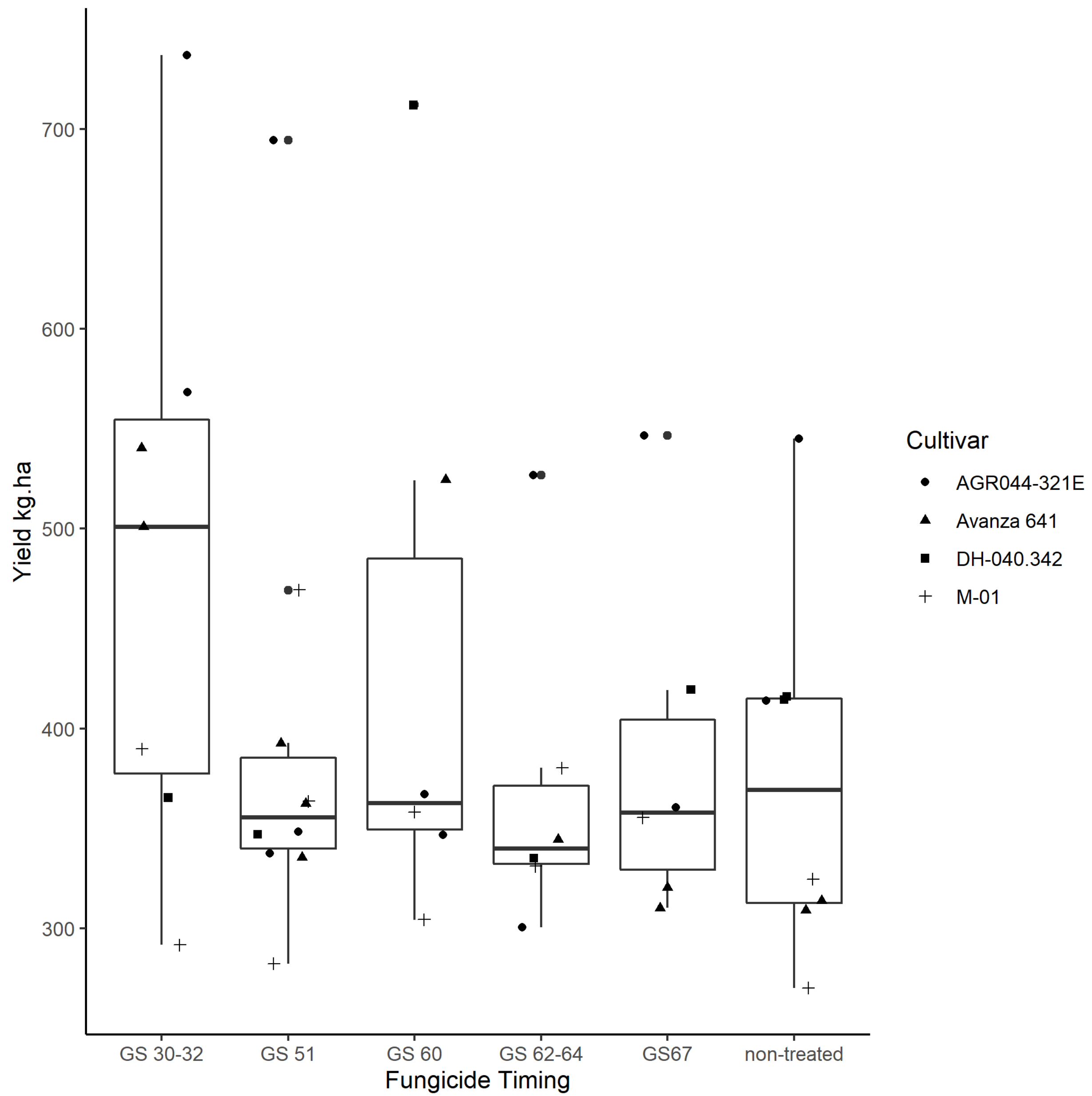

Fig. 4.5. 2022 *B. carinata* yield for each fungicide timing in the 2021-2022 season.

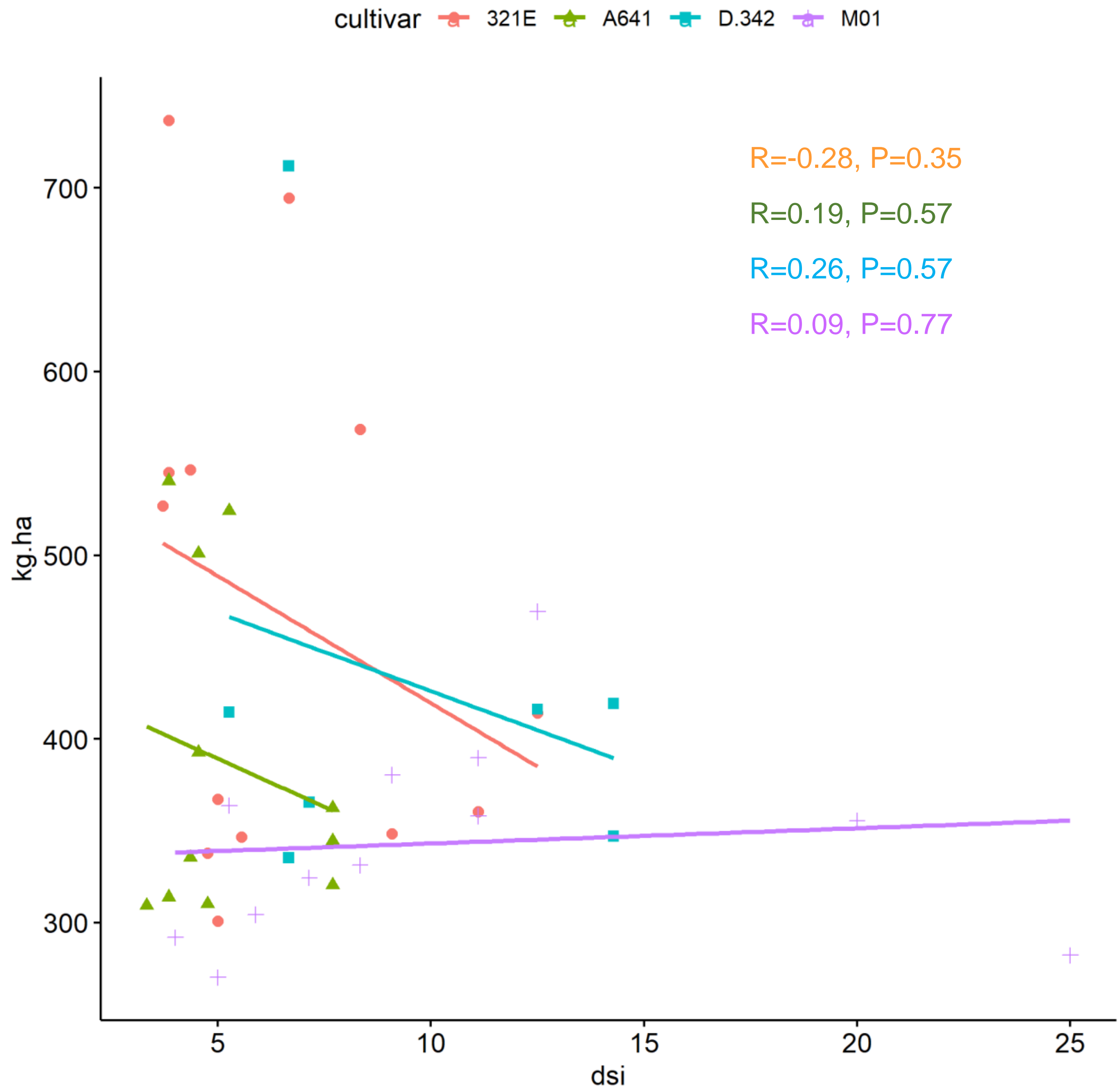

Fig. 4.6. 2022 B. carinata yield plotted against disease severity index, separated by cultivar. Fit statistics also shown.

Table 4.2 Cultivars, fungicide timings, and interactions of the two that were significantly different in yield of B. carinata in the 2021/2022 production season.

Cultivar	Fungicide Timing	P value
DH-040.342	-	0.042
M-01	-	0.002
-	GS 51	0.035
-	GS 60	0.002
-	GS 62-64	0.009
-	GS 67	0.040
M-01	GS 51	0.050
AVANZA 641	GS 60	0.027
DH-040.342	GS 60	<0.001
M-01	GS 60	0.023
M-01	GS 62-64	0.040

V. In vitro Fungicide Efficacy Against Sclerotinia sclerotiorum

Abstract

Sclerotinia sclerotiorum is a major pathogen of over 400 plant species around the world and is considered to be the biggest threat to the production of *Brassica carinata* in the southeastern United States. The present study seeks to test the *in vitro* efficacy of Caramba (metconazole 89.87g/L), Sphaerex (metconazole 112.52g/L + prothioconazole 187.53 g/L), Endura (boscalid 674.86g/L), and Quadris (azoxystrobin 249.24g/L) on southeastern US isolates of *S. sclerotiorum*. Isolates were BR (Blairesville, GA), TI (Tifton, GA) and QC (Quincy, FL). All tested fungicides significantly inhibited growth; however, Quadris inhibition was significantly lower than all other fungicides at every concentration, for nearly every isolate.

Introduction

Sclerotinia sclerotiorum is the causal agent of Sclerotinia stem rot (SSR); a major disease of *B. carinata* as well as *Glycine max* (soybean), *Brassica napus* (canola), *Pisum sativum* (field pea) (Boland and Hall 1994) and over 400 other plant species around the world (Heffer Link and Johnson 2007). Typical symptoms of *S. sclerotiorum* infection include wilted leaves and bleached stems; signs include fluffy white mycelium and sclerotia formed on and inside dead plant tissue (Heffer Link and Johnson 2007). While *B. carinata* is reported to be moderately tolerant to *S. sclerotiorum,* there are no known resistant cultivars (Yang et al. 2009). This necessitates the implementation of cultural practices and chemical controls which can reduce incidence and severity of sclerotinia stem rot.

The North Dakota State University plant disease management guide lists Endura and Quadris for control of *S. sclerotiorum*. Quash (metaconazole) is included on this list, but

Caramba is not. Sphaerex does not have a counterpart with both active ingredients; however, Proline (prothioconazole) and Quash (metconazole) are listed. Zamani-Noor (2021) tested boscalid, prothioconazole, and azoxystrobin (among other chemical and biological controls) against different *S. sclerotiorum* isolates collected in Germany and found boscalid to have the highest fungicidal activity *in vitro.* Azoxystrobin and prothioconazole were reported to have the most variable fungicidal activity between isolates. Metconazole had high activity against *S. sclerotiorum* collected in different regions of the Henan Province of China (Liu et al. 2021).

The present study seeks to test the *in vitro* efficacy of Caramba (metconazole 89.87g/L), Sphaerex (metconazole 112.52g/L + prothioconazole 187.53g/L), Endura (boscalid 674.86g/L), and Quadris (azoxystrobin 249.24g/L) on southeastern US isolates of *S. sclerotiorum*.

Materials and Methods

Fungal Isolates and Storage

The *S. sclerotiorum* isolates were named according to their source location: Blairesville, GA (BR), Brewton, AL (BT), Tifton, GA (TI), and Quincy, FL (QC). Isolates sourced by a former lab member who collected sclerotia from *B. carinata* fields in each location. These sclerotia were stored at room temperature for an unknown period in coin envelopes prior to use in this experiment. It is likely that these sclerotia did not originate from the field but were generated from original sclerotia according to Pethybridge et al. (2015) by the former lab member. The inoculum for this experiment was generated by incubating these sclerotia on potato dextrose agar plates (PDA) at room temperature (19-21°C) and reculturing subsequent plates with 7mm mycelial plugs.

In vitro Efficacy Experiment

This experiment was conducted twice by preparing potato dextrose agar (PDA) (various manufacturers) according to manufacturer instructions. Following autoclaving, media was cooled to approximately 60°C and amended with one of four fungicides including Caramba (metconazole 89.87g/L), Sphaerex (metconazole 112.52g/L + prothioconazole 187.53 g/L), Endura (boscalid 674.86g/L), and Quadris (azoxystrobin 249.24g/L) at concentrations of 2%, 5%, 10%, 50%, and 100% maximum recommended label rate (MRLR). These concentrations were calculated by converting the area of a 15 x 100 mm petri dish, which contained 20 mL of PDA, to acres (for more information of fungicide amendments see Table 5.1). Tested *S. sclerotinia* isolates were BR (Blairesville, GA), TI (Tifton, GA) and QC (Quincy, FL).

Amended PDA plates, as well as unamended controls, were each inoculated with a 7mm plug of four-day old mycelium. Plates were incubated at room temperature (19-21°C) for 72 hours, with colony diameter recorded every 24 hours. There were five replicates of each treatment except for isolate BR which had three replicates for non-control plates in trial 1. The experiment was done twice.

Percent of inhibition was calculated by comparing the diameter of treated plates to the mean diameter of the control plates with a corresponding isolate.

Data were analyzed in R (R version 4.0.3; 2020-10-10). Analysis of variance of the effects of repetition, isolate, fungicide, and the interaction of these variables on inhibition was conducted to determine if the two trials were significantly different from each other at each concentration.

Significant difference in mean inhibition by isolate, fungicide, and the interaction of isolate and fungicide was determined via analysis of variance. This was followed by a Tukey's test which compared the inhibition means of all fungicide-isolate interactions to each other. The multcompview package was used to add compact letter display (CLD) to the Tukey's test results, which added different letters to means that were significantly different from each other. Finally, bar plots showing the mean inhibition of each fungicide on each isolate as well as CLD was created using the ggplot2 package. Each concentration was analyzed separately to allow for missing data to be subset from smaller sections rather than the entire analysis.

Results

There was a significant interaction effect of the experimental repeat with the treatments on fungal growth inhibition. As a result, each trial was analyzed separately.

Trial 1

Endura was removed from analysis in the 2% and 5% concentrations due to experimental error. Similarly, Caramba was removed in 10% and 100% concentration analysis. Errors included *pencillium* spp. contaminations, failure to inoculate certain fungicide concentrations, and dropping a stack of inoculated plates which dislodged inoculum plug and altered colony growth.

Analysis of variance showed significant differences in mean inhibition between fungicide, isolate, and the interaction of fungicide and isolate for concentrations 2% and 5%. The remaining concentrations only saw significant difference in means between fungicides (Fig. 5.1). Sphaerex consistently had the highest inhibition among all three

isolates, at every concentration. Conversely, Quadris consistently had the lowest inhibition among all isolates at all concentrations.

Trial 2

Isolate QC was mistakenly used to inoculate plates designated as BR as well as plates designated as QC; resulting in isolate QC having 10 replications at 10% fungicide concentration Caramba and BR being omitted. BR was omitted from data analysis at 10% concentration for all fungicides to allow for pairwise comparison between remaining isolates.

Significant differences were seen between fungicide, isolate, and the interaction of fungicide and isolate for all concentrations (Fig. 5.2). At 2% concentration, Endura had significantly higher inhibition than all other fungicides. Sphaerex had the highest inhibition in all isolates in all remaining concentrations other than BR 100%, which was more inhibited than by Caramba; however, these treatments were not significantly different from each other. Quadris had the lowest inhibition for all isolates at all concentrations.

Discussion

Due to some fungicides not being tested at all concentrations, it is difficult to definitively state their relative effectiveness. However, among all concentrations, in both trials, Quadris had the lowest inhibition and was frequently, significantly lower than the inhibition of all other fungicides. In most cases, especially at higher concentrations, Sphaerex and Caramba all exhibited high levels of inhibition and were not significantly different from each other. Endura was frequently also not significantly different, however

sufficient comparisons to sphaerex and caramba cannot be drawn from the results of this study.

The significant difference between trials of this experiment may be due to a number of factors including fluctuation in room temperature during the incubation period, the use of various PDA brands, PDA being maintained at molten state for various lengths of time (2-24 hours), or difference PDA temperature at the time of amendment. Future studies should standardize these variables.

According to the 2022 FRAC code list, metconazole and propconazole are at medium risk of resistance development, boscalid is medium-high, and azoxystrobin is listed as high risk for resistance development. Resistance must be carefully managed in these FRAC groups since they have single site modes of action rather than multisite, which means pathogens only have to develop a single resistance gene. In a study on boscalid resistance in southwest China, a single *S. sclerotiorum* isolate (of 399 tested) was found to be resistant. (Li et al. 2022). It was supposed that resistance was due to random variation rather than selection pressure given limited number of applications and short history of boscalid use in the region. While resistance to metconazole, propiconazole, and azoxystrobin has not yet been reported in *S. sclerotiorum* it has been reported in other pathogens. For example, isolates of *Pyrenophora teres* have been reported to be resistant to metconazole (Mair 2016), isolates of *Sclerotinia homeocarpa* have been reported to be resistant to propiconazole (Golembiewski 1995), and azoxystrobin failed to control resistant isolates of *Colletotrichum acutatum* (Forcelini 2016).

The results of this study indicate that the tested *S. sclerotinia* isolates may be resistant to azoxystrobin, however further study is required to confirm. It has been reported that

some fungicides are ineffective in field tests when disease pressure is high (Mueller et al. 2002). Therefore, a future study should employ these fungicides on *B. carinata* fields to test for differences in severity, incidence, and yield.

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Tables and Figures

Table 5.1 Additional information on fungicides tested for in vitro against S. sclerotiorum.

Trade Name	Active Ingredient	Company	FRAC Group	a.i. g/L	Fungicide per L of Media
Caramba	metconazole	BASF	3	89.87	48.72 µL
Sphaerex	metconazole + prothioconazole	BASF	3 + 3	112.52 + 187.53	20.92 µL
Endura	boscalid	BASF	7	674.86	0.03 g/L
Quadris	azoxystrobin	Syngenta	11	249.24	44.42 µL

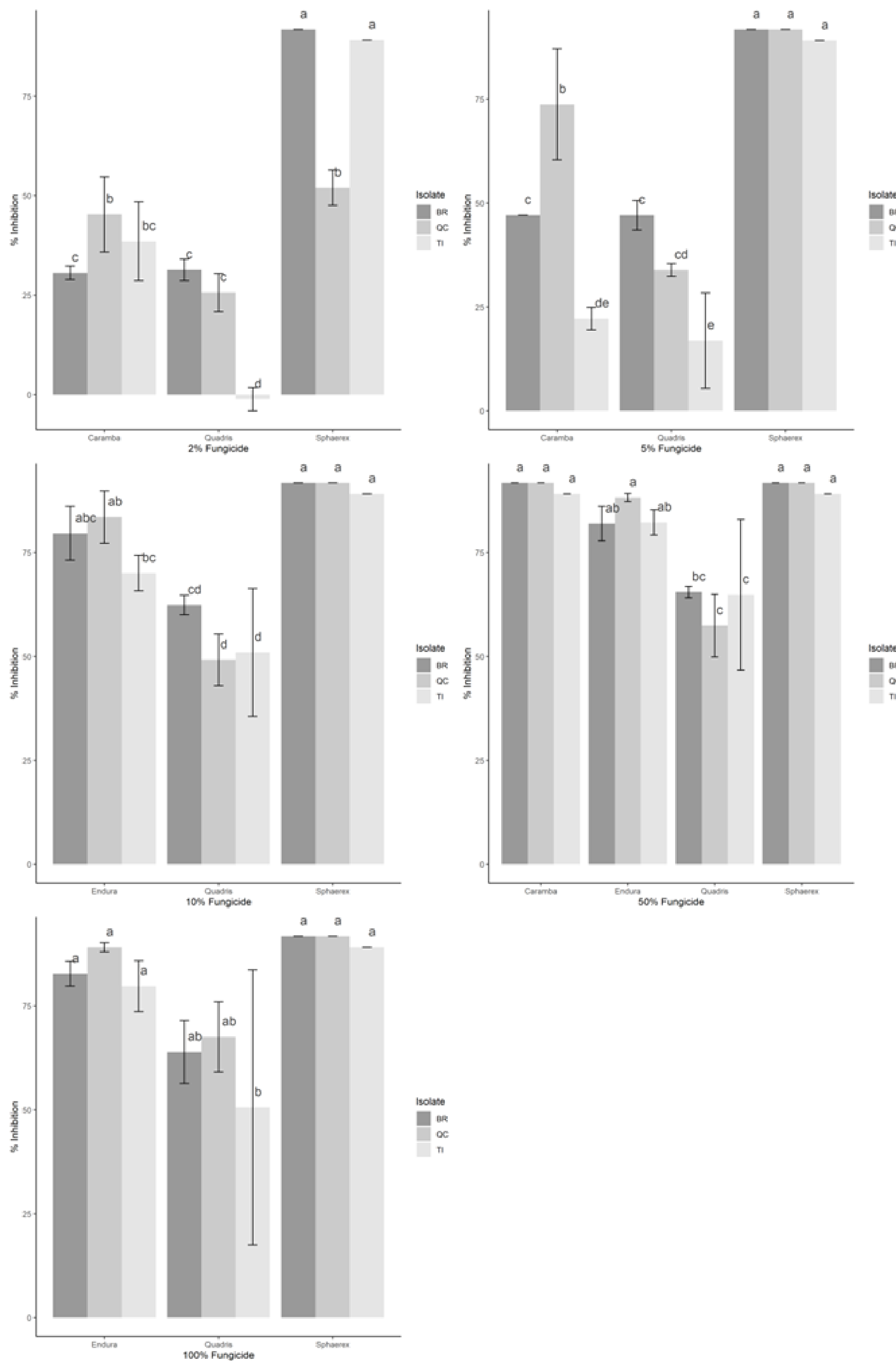

Fig. 5.1. Mean inhibition for each fungicide and isolate for the first trial, at each fungicidal concentration. Treatments with the same letter in each concentration are not significantly different from each other, according to Tukey's test.

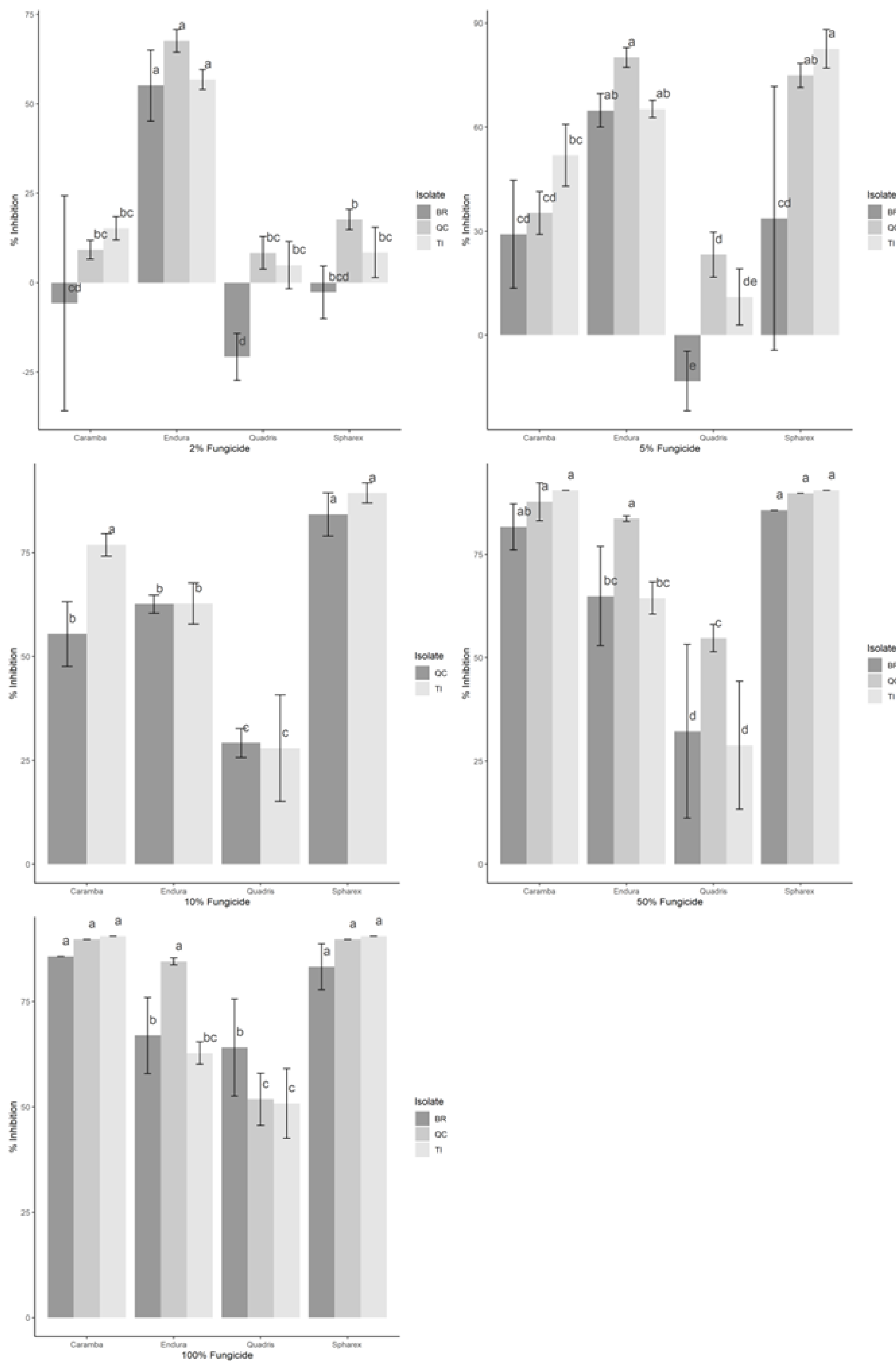

Fig. 5.2. Mean inhibition for each fungicide and isolate for the second trial for each fungicidal concentration. Treatments with the same letter in each concentration are not significantly different from each other according to Tukey's test.