Novel Environmentally-Friendly Alternatives to Conventional Fungicides in Combating Bitter Rot (*Colletotrichum gloeosporioides*) in Idared and Golden Delicious Apples (*Malus domestica*)

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Abstract

Bitter Rot (*Colletotrichum gloeosporioides*) is one of the most destructive diseases affecting the apple crop around the world. With consistently high crop production required to meet demand, disease management is important for farmers to not only support themselves, but to support everyone around them. There have been signs of fungicide resistance to demethylation-inhibiting fungicides, the standard fungicides used to treat Bitter Rot commercially (Gao et al. 2009). Conventional fungicides have also been shown to be harmful to the environment, leading to surface runoff and volatilization (Scitable). 8 novel environmentally-friendly treatments were tested as alternatives to the conventional fungicide program, such as citrus extract, plant defense activators, white mineral oil, and organic fungicides. If alternative methods of control are not considered, food security could be threatened in the future as crop output will decrease from increased fungicide resistance. Furthermore, disease levels will increase in pressure due to the warmer temperatures associated with climate change, since fungi would have greater overwintering survival rates (Chaloner 2021). Data on the disease incidence of the apple fruit were analyzed using the LSD and Tukey's tests under ANOVA. Disease incidence was higher due to greater than average precipitation (Fig. 7). Incidence ranged from 5.76% to 48.19% for Golden Delicious and 11.94% to 88.67% for the Idared variety (Fig. 9). The most effective treatment overall for both the Idared and Golden Delicious varieties was Omega 500. This study provides valuable information on the potential of environmentally-friendly methods of disease control. Further research needs to be conducted to evaluate the efficacy of these environmentally-friendly treatments in various climates, temperatures, and regions.

Introduction

Every year, the average American consumes about 45.2 pounds of apples ("Apple Facts"), making apples the most consumed fruit in the United States ("Apples and Oranges Are the Top U.S. Fruit Choices"). Production levels are heavily influenced by farmers' ability to manage diseases, especially in top apple-producing states such as Virginia, which ranks 6th in apple production ("Virginia Apple Page -Virginia Tech's Site for Apple IPM and Production"). Virginia is affected by about 10 fungal apple diseases, including Bitter Rot. Bitter Rot (*Colletotrichum gloeosporioides*) is one of the most devastating diseases to commercial apple growers around the world. The most destructive of the three types of rots that affect apples (bitter rot, white rot, and black rot) and common to warmer growing regions, Bitter Rot is especially damaging to Virginia during its hot, humid summers. Known for its signature circular spot filled with conidia swirls in the middle of an apple, it is unable to be sold once evidence of the disease marks a piece of fruit. Thus, Bitter Rot, among other crop diseases, not only threatens the livelihoods of farmers, but also the vitality of food production. More efficient control of Bitter Rot can increase yields and strengthen food security for generations to come.

Fig. 1 Two apples infected with Bitter Rot in Winchester, VA

There has been evidence of fungal resistance in commercial orchards as a result of successive applications of demethylation-inhibiting fungicides (Gao et al. 2009), which are the standard fungicides currently used. Therefore, fungicide resistance reduces the efficacy of fungicides. Farmers' profits could be affected in the future by this pressing issue.

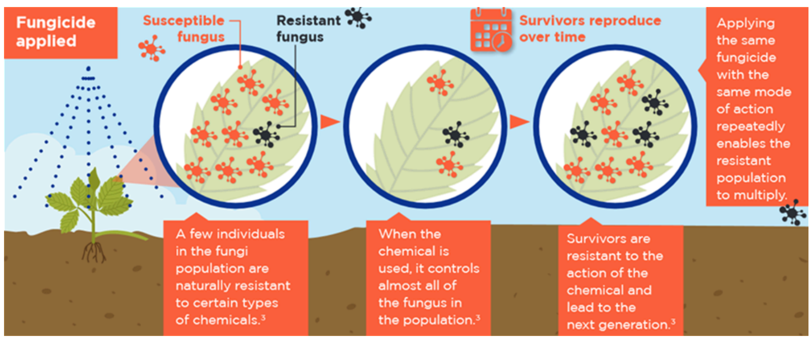

Fig. 2 How Fungicide Resistance Develops in an Orchard (Fungicide Resistance Action Committee)

In addition, conventional fungicides pose hazards to wildlife and the environment. They contribute to surface runoff in rivers and streams, groundwater leaching, a serious danger for those who rely on groundwater as a drinking water source, and volatilization, when the vapor form of the chemical spreads to wildlife areas (Scitable)(Fig. 3). The application of fungicides has also led to a decrease in enzyme activity and the bacteria populations in the soil (Roman et al. 2021). Soil bacteria allow a multitude of plant species to survive, develop strong structures, and battle pathogens (Hayat et al. 2010). The unsustainability of current practices demand a search for more environmentally-friendly alternatives.

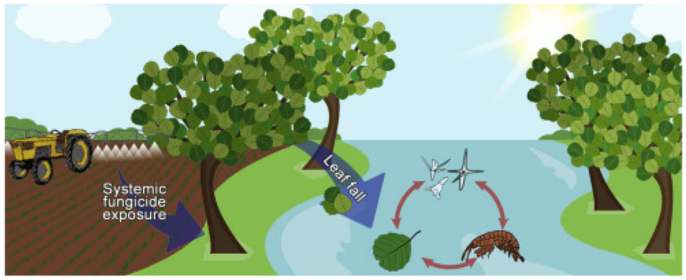

Fig. 3 Dangers of conventional fungicides (Newton et al. 2018)

While cultural practices, such as removing diseased fruit from commercial orchards are beneficial in reducing the spread of Bitter Rot from year to year, they cannot be the sole remedy (Sutton 2014). Fungicides are the most effective method of disease control and guarantee the highest yield and profit for farmers. Therefore, it was imperative that this research aimed to study the efficacy of a mixture of different types of fungicides, including plant activators, mineral oil, citrus extract, organic fungicides, and alternative fungicides.

Plant defense activators are a newer group of chemicals that activates and aids the plant defense system rather than directly harming the pathogen (Walter et al. 2005). Some activators stimulate proteins involved with pathogenesis or activate dormant defense genes (Reddy 2012). However, little is known about their effectiveness in disease control against Bitter Rot, and how this might differ based on the variety tested (Verly et al. 2020).

Golden Delicious and Idared are among the most popular apple varieties sold in the United States (“Varieties Archive - New York Apple Association”). The Golden Delicious variety is known for its deep yellow exterior and crunchy taste, while the Idared variety is a multi-purpose red-green fruit. These cultivars were selected for this study because of their differing harvest stages. The timing of the harvest stage is significant because certain varieties display signs of disease earlier than others. For example, the Golden Delicious variety displays Bitter Rot later on during the season, but since it is harvested earlier, yield is less likely to be affected than if a variety displays the disease earlier in the season. Less fruit can be sold if the disease is displayed earlier in the season. Therefore, the results of this study would be more applicable to commercial apple orchards than if lesser known varieties or if only one harvest timeline was tested.

Purpose

The purpose of this study was to assess the efficacy of 19 different fungicide treatments in the control of Bitter Rot (*Colletotrichum gloeosporioides*) in Golden Delicious and Idared apples. Specifically:

1. Comparing the disease incidences of Bitter Rot in Golden Delicious and Idared apples
2. Comparing the efficacy of 4 different plant activators against Bitter Rot
3. Evaluating the efficacy of environmentally-friendly methods of control against Bitter Rot

Hypotheses

1. The grower standard (conventional treatment) will be the most effective treatment because no environmentally-friendly treatment has been found of yet that is more effective in treating Bitter Rot than the conventional treatment.
2. The Golden Delicious variety will be infected less severely with the Bitter Rot disease than the Idared variety.

Methods

Research Design and Site

This study took place in an experimental orchard with 22 year old Golden Delicious and Idared apple trees (M.111 rootstocks). There were 30 feet between rows, 14 feet between trees in a set, and 28 feet between each plot. The plot was arranged in pairs, with one Golden Delicious tree and one Idared tree next to each other. There were 4 replicates per treatment. The plot consisted of 4 blocks to evaluate whether or not moisture, temperature, and other environmental conditions affected disease incidence. Most orchards consist of a sloping landscape, therefore causing areas at the bottom of the slope to build up in moisture. With an increased level of moisture, the fungus can more efficiently spread.

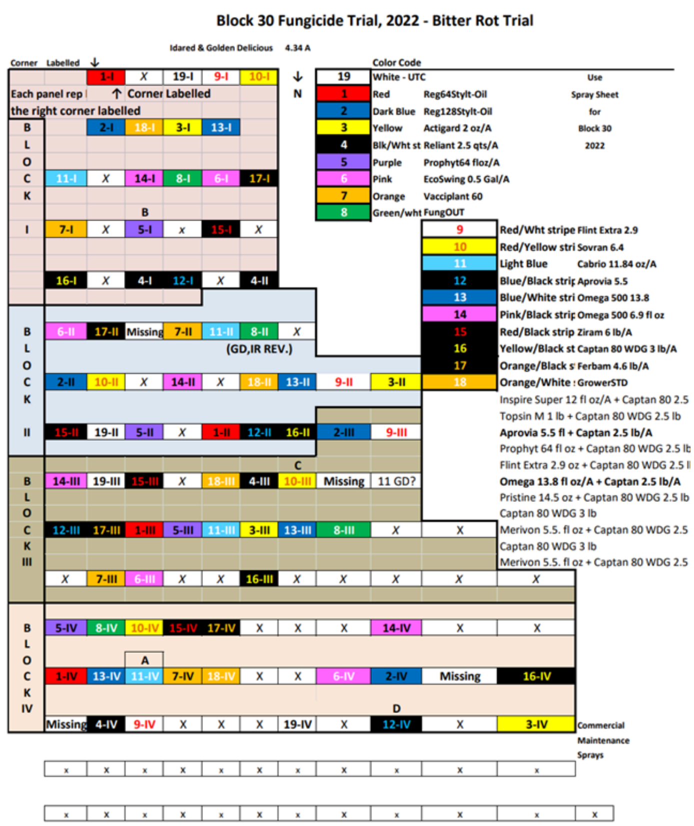

Fig. 4 Field Plot Design

Fungicide Treatments

20 treatments were applied in this study by the farm manager. This included 8 environmentally-friendly solutions: 2 concentrations of Stylet Oil (white mineral oil), the Actigard biological activator, the Reliant plant activator, the ProPhyt plant activator, Ecoswing (extract of citrus *Swinglea glutinosa*), Vacciplant (brown algae plant activator), and FungOUT (organic fungicide). The eight alternative fungicides to combat fungicide resistance included: Flint Extra, Sovran, Cabrio, Approvia, Omega, Ziram, Captan, and Ferbam. GrowersSTD, the conventional treatment plan to treat Bitter Rot was applied, as well as an un-inoculated untreated treatment and an inoculated untreated treatment (Fig. 7). Fungicides were applied every two weeks or after at least 2 inches of rainfall according to recommended dosages. 8 cover sprays were applied in total.

Maintenance sprays to treat against diseases besides Bitter Rot were applied 6 times throughout the study by the farm manager. These were applied so that the chance of other diseases affecting and weakening the trees would be lessened. Thus, I would get a more accurate representation of the incidence of Bitter Rot (Fig. 6).

Equipment utilized to apply the treatments and maintenance sprays included: a sprayer on top of a tractor with a Friend pecan brass spray gun #16 (nozzle #12 Pak-Tank 4 x 25-gal sprayer, 250 PSI, Rear's Manufacturing, Coburg, OR) with a dilute drip (400 gal/A). The spray flow was 11.7 gal/min.

Spray Dates	Programs
5/23/2022	3C
6/7/2022	Inoculation
6/8/2022	4C
6/21/2022	5C
6/25/2022	6C (after 2" rain event on 23 June)
7/8/2022	7C
7/22/2022	8C

Fig. 5 Dates of treatment applications. C = cover spray

Dates	Treatments
3/29/2022	Vanguard 5oz/A + Mancozeb 3lb/A + Bio-Cover Oil 2 Gal/100 gal
4/6/2022	Inspire Super 12 fl oz/A + Manzate Pro-Stick 3 lbs/A + Assail 4 oz/A
4/21/2022	Inspire Super 12 fl oz/A + Manzate Pro-Stick 3 lbs/A + Sonoma 10 oz/A
5/4/2022	Inspire Super 12 fl oz/A + Microthiol Disperss 7 lb/A + Manzate Indar 8
5/28/2022	Altacor 4.5 oz/A; 6/28/2022: Imidan 5 lb/A + Assail 4 oz/A
6/28/2022	Imidan 5 lb/A + Assail 4 oz/A
7/15/2022	Voliam Flexi 5 oz/A

Fig. 6 Maintenance Sprays and dates applied

Table 1. Treatments for control of apple bitter rot evaluated in 2022 allowing to compare natural and alternative fungicides to synthetic fungicides.

#	Spray materials and rate	Active ingredient (FRAC code, Mode of Action)	Application stage/ timing*	Spray interval
1	Regalia 64 fl oz/A + JMS Stylet-Oil 1 gal/100 gal	extract from *Reynoutria sachalinensis* (P05, anthraquinone elicitor)	3rd to 9th cover spray*	14 days or 2 inches of rain, whichever comes first, but if no rain occurred for 14 days, extend spray interval to 21 days, under the condition that we do not get rain during the 7 additional days. If any rain event occurs between 14 and 21 days, apply fungicide before that rain regardless was 21 days reached or not.
2	Regalia 128 fl oz/A + JMS Stylet-Oil 1 gal/100 gal			
3	Actigard 2 oz/A	acibenzolar-*S*-methyl (P01, SAR activator)		
4	Reliant 2.5 quarts/A	(P07, phosphonates)		
5	Prophyt 64 fl oz/A			
6	EcoSwing 0.5 Gal/A	extract of *Swinglea glutinosa* (BM01, affects fungal spores and germ tubes, induced plant defense)		
7	Vacciplant 60 fl oz/A	laminarin (P04, polysaccharide elicitor)		
8	FungOUT 3.75 gal/A	1.07% citric acid (NA**)		
9	Flint Extra 2.9 fl oz/A	trifloxystrobin (11, QoI)		
10	Sovran 6.4 oz/A	kresoxim-methyl (11, QoI)		
11	Cabrio 11.84 oz/A	pyraclostrobin (11, QoI)		
12	Aprovia 5.5 fl oz/A	benzovindiflupyr (7, SDHI)		
13	Omega 500 13.8 fl oz	fluazinam (29, UOPP)		
14	Omega 500 6.9 fl oz	fluazinam (29, UOPP)		
15	Ziram 6 lb/A	ziram (M03, multisite)		
16	Captan 80 WDG 3 lb/A	captan (M04, multisite)		
17	Ferbam Granuflo 4.6 lbs/A)	ferbam (M03, multisite)		
18	**Grower Standard** • Inspire Super 12 fl oz/A + Captan 80 WDG 2.5 LB/A • Topsin M 1 lb + Captan 80 WDG 2.5 lb • Topsin M 1 lb + Captan 80 WDG 2.5 lb • Prophyt 64 fl oz + Captan 80 WDG 2.5 lb • Flint Extra 2.9 oz + Captan 80 WDG 2.5 lb • Flint Extra 2.9 oz + Captan 80 WDG 2.5 lb	difenoconazole (3, DMI) + cyprodinil (9, AP) + captan (M04) thiophanate-methyl (1, MBC) + captan (M04) thiophanate-methyl (1, MBC) + captan (M04) potassium phosphite (P07, phosphonates) + captan (M04) trifloxystrobin (11, QoI) + captan (M04) trifloxystrobin (11, QoI) + captan (M04)		
19	Untreated inoculated control	-	-	-
20	Untreated non-inoculated control	-	-	-

*Note: The treatments were initiated after primary apple scab season is over and will start from first cover or third cover spray and will continue until ninth cover spray if needed, or until disease incidence data is collected.
**NA – FRAC code not yet known and/or assigned.

Fig. 7 List of treatments used in the study

Fruit Inoculation

My mentor and his graduate student inoculated Golden Delicious apples at 25mm in the laboratory using mycelial plugs of *C. fruticola*. Then, they incubated the fruit in the dark at 77°F until signs of Bitter Rot began to form: after around 15 days. Afterwards, they placed the fruit into mesh produce bags and hung them at the top of every tree involved in the study on June 7, 2022.

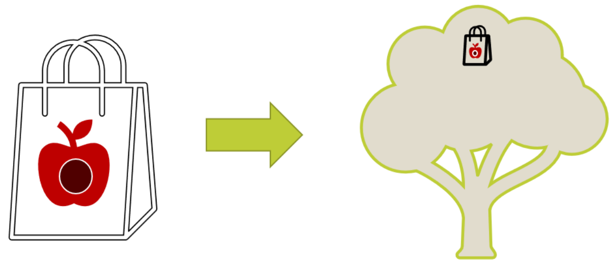

Fig. 6 Fruit inoculation diagram

Disease Rating

Bitter Rot Incidence was measured twice during the summer: from July 25-26, 2022 and another from August 1-5, 2022. The trial was measured twice to quantify the increase in Bitter Rot incidence from the first date of Bitter Rot observation to later on the season. My mentor and his graduate student collected data for the initial measurement and his graduate student and I collected data for the second measurement. Fifty fruit per Golden Delicious and Idared apple tree were selected and diagnosed for symptoms of Bitter Rot as a part of each measurement of Bitter Rot Incidence. The number of infected fruit vs the total number of fruit were recorded for each treatment and replicate. Any trees within the plot that did not produce at least 50 apples were not included in this study.

Data Analysis

I processed the data, calculating the mean of the disease incidence for each treatment repetition cluster, each treatment repetition, and then each treatment. Then, I graphed the data into histograms. The data was analyzed under the LSD test ($\alpha = 0.05$) for the Idared apple disease incidences and the Tukey's test (α = 0.05) for the Golden Delicious apple incidences by my mentor under a completely randomized design to evaluate whether there was a significant difference between the efficacy of each treatment. This was because the 4 blocks established in the plot were not found to have significant differences in resulting disease incidence due to miscellaneous environmental conditions (moisture, temperature, etc.). Many orchards do not have flat landscapes, leading to a buildup of moisture at the bottom of slopes. Lastly, I formulated conclusions on the efficacy of each treatment based on the data my mentor analyzed.

Weather Data

Weather data was collected using the Rimpro database by my mentor to measure the temperature, precipitation, and level of infection throughout the study (April-mid August). It was found that the amount of rainfall over the course of the growing season was higher than the average, providing the moist conditions that Bitter Rot fungi thrive in. Thus, 6 major infection periods were found to have occurred. Environmental conditions can heavily affect the fungicide treatment plans farmers have to apply to their crops. When a specific rainfall event is greater than 2 inches, a fungicide application needs to be reapplied. Therefore, it was imperative that we took note of the context of the Bitter Rot disease levels (Fig. 7).

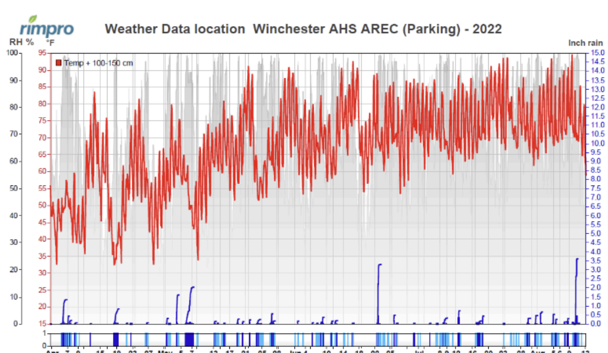

Figure 1. Summary of weather conditions in 2022 during the apple bitter rot trial at Winchester, VA with multiple rain events and above average amount of rain, that allowed numerous disease infection periods for apple bitter rot. Source: RIMpro B.V., France, subscription-based service.

Fig. 7 Weather conditions throughout the study

Results

July 26, 2022 Data

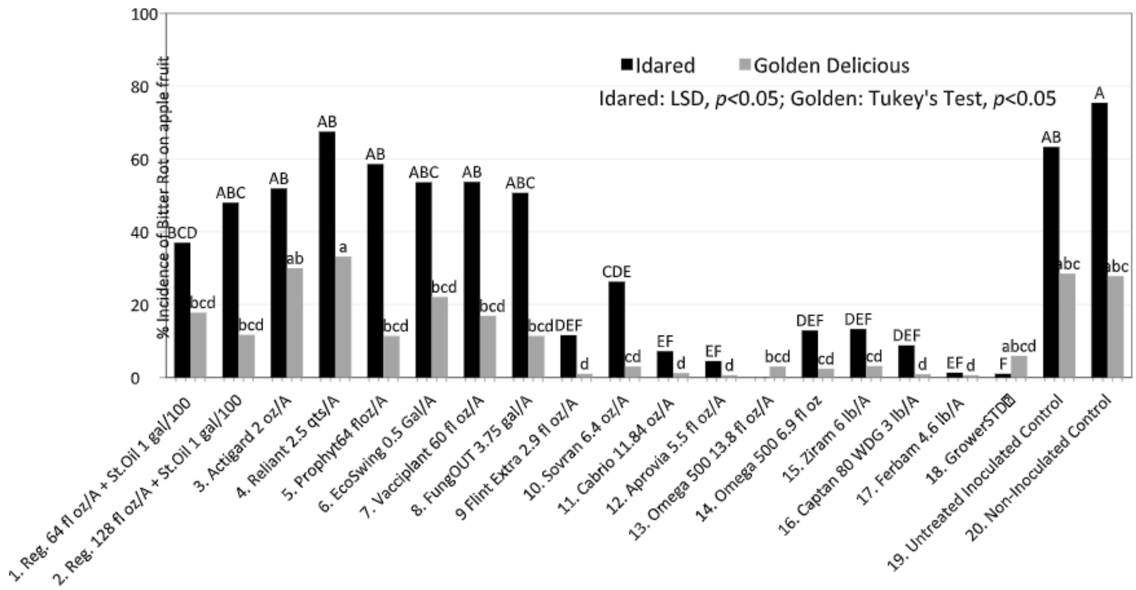

Fig. 8 July 26, 2022 disease incidence results

Disease levels of Bitter Rot were high due to increased precipitation (Fig. 7). Thus, the uninoculated control treatment had almost an 80% disease incidence and the untreated inoculated control had around a 60% disease incidence in the Idared apple variety (Fig. 8). In the Golden Delicious variety, the uninoculated control treatment and the untreated inoculated control treatment had similar resulting disease incidences- around 30%. The conventional treatment, otherwise known as the Grower Standard, was the most effective treatment on the Idared Apple, causing less than a 5% disease incidence. The best treatments for the Golden Delicious variety were Captan, Approvia, Cabrio, and Flint Extra. Overall, the Ferbam 4.6 lb/A was the most effective treatment overall. Of the 8 environmentally-friendly treatments, Reg 64 fl oz/A + Stylet

Oil was the most effective treatment for the Idared variety. The least effective treatments were Actigard, Reliant, and Prohyt64 for the Idared variety. For the Golden Delicious Variety, the Reg. 64 fl oz/A + Stylet Oil, the Reg. 128 fl oz/A + Stylet Oil, Prophyt64, Ecoswing, and Vacciplant treatments were the most effective of the environmentally-friendly treatments, resulting in similar incidences of around 10-15%. The Reliant 2.5 qts/A treatment was the least effective of the environmentally-friendly fungicides.

August 5, 2022

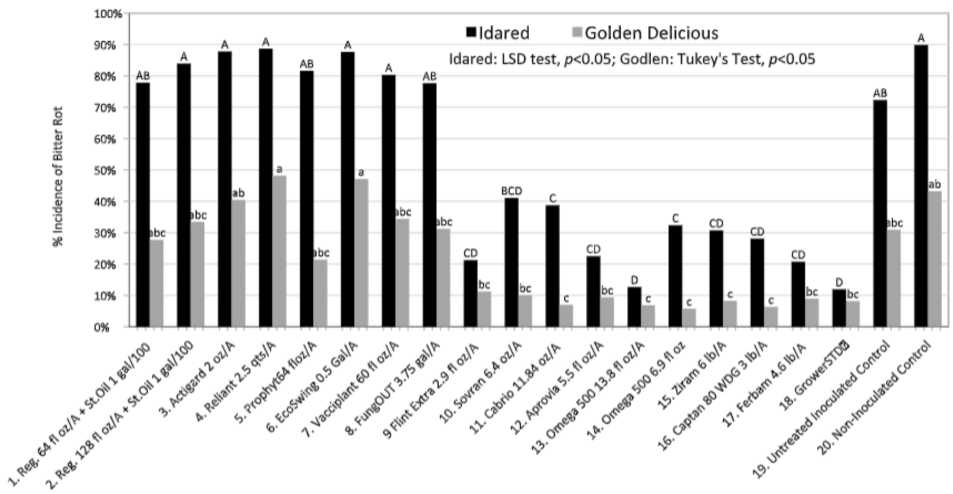

Fig. 9 August 5, 2022 disease incidence results

The disease incidence of Bitter Rot increased on August 5, 2022. The untreated uninoculated control had a 70% incidence and the non-inoculated control had a 90% incidence for the Idared apple variety. For the Golden Delicious apple variety, there was a 30% incidence for untreated inoculated control and about a 45% incidence for the non-inoculated control. The most effective treatments for the Idared apple variety were Omega 500 13.8 fl oz/A and the Grower Standard. The least effective treatments were Reg. 128 fl oz/A, Actigard, Reliant, EcoSwing, and Vacciplant. The most effective treatments for the Golden Delicious variety were

Cabrio, Omega 500, Ziram, and Captan. The least effective treatments were Reliant 2.5 qts/A and EcoSwing. The most effective treatment overall was Omega 500 13.8 fl oz. There was no significant difference in the efficacy of the environmentally-friendly treatments.

Discussion/Conclusion

An increase in the disease incidence of Bitter Rot was observed overall from July 26, 2022 to August 5, 2022 by an average of 29.5% for the Idared variety and 10.6% for the Golden Delicious variety. The untreated non-inoculated and inoculated treatments resulted in high incidences of disease due to higher than average precipitation in the spring. This increase in moisture due to the precipitation encouraged the spread of the fungi throughout the growing season. Disease incidence ranged from 5.76% to 48.19% for Golden Delicious (August) and 11.94% to 88.67% for the Idared variety (August). The Reg64 Stylet oil was more effective than the Reg128 Stylet oil. This study gives insight into the effectiveness of 8 novel environmentally-friendly fungicides. The most effective treatment was Omega 500.

Food Security

This study is also relevant to food security. If alternative fungicides are not explored for potential commercial usage, fungicide resistance will continue to be an ever-pressing problem that could eventually reduce agricultural productivity, threatening the security of our food system. In many developing countries around the world, obsolete fungicides are applied. These dangerous conditions are made worse in many areas that lack the personal protective equipment needed to safely apply these chemicals. This issue has led to around 385 million cases of toxic pesticide poisoning across the globe (Boedeker 2020). Further, since the warmer temperatures associated with climate change will increase the survival rate of fungi, disease levels will only increase over time. Therefore, this study is valuable not only to current farmers, but also to the next generation as a step toward ensuring that our food systems are sustainable.

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