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Effects of Bacillus Mycoides Supplement in a Reduced Frequency Fungicide Program on Chambourcin Grapevines (Vitis Vinifera L.)

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EFFECTS OF *BACILLUS MYCOIDES* SUPPLEMENT IN A REDUCED FREQUENCY FUNGICIDE PROGRAM ON CHAMBOURCIN GRAPEVINES (*Vitis vinifera* L.)

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Master of Science

By
Ryan Alan Mairs

August 2018

EFFECTS OF *BACILLUS MYCOIDES* SUPPLEMENT IN A REDUCED FREQUENCY FUNGICIDE PROGRAM ON CHAMBOURCIN GRAPEVINES (*Vitis vinifera* L.)

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I dedicate this thesis to my wife Morgan, who has been a constant support to me throughout my entire academic career. Also to my family and friends who have encouraged me to follow the path that has lead me into higher education and enriched my life as a result. Lastly to my Grandpa George Austin; A man who farmed all his life and taught me that wisdom is acquired in different ways. I know you would be proud of me if you were still here today.

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EFFECTS OF *BACILLUS MYCOIDES* SUPPLEMENT IN A REDUCED FREQUENCY FUNGICIDE PROGRAM ON CHAMBOURCIN GRAPEVINES (*Vitis vinifera* L.)

Ryan Mairs August 2018 25 Pages

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Fungal diseases pose significant challenges for grapevine producers in Kentucky due to the region's abundant moisture and relative humidity. Methods to reduce fungicide application frequency would prove both economically and temporally valuable to producers. A field experiment was established in Bowling Green, KY in 2017 to investigate *Bacillus mycoides* isolate J (LifeGard™) as a supplement to a fungicide program for systemic acquired resistance (SAR). Three fungicide treatment regimens were implemented consisting of a program modelled from the Midwest Fruit Pest Management Guide (2017) and an identical program supplemented with 140 g ha^{-1} LifeGard™ per application (both applied on 14 day intervals), a reduced frequency application every 28 days supplemented with 140 g ha^{-1} LifeGard™, and an untreated control. Treatments were applied to 9-year-old French-Hybrid grapevines (cv. Chambourcin); each treatment was replicated 3 times in a randomized complete block design. All treatments were applied with a backpack sprayer delivering 150 L ha^{-1} at 2 Bar pressure. Canopy management, fertility, herbicide, and insect management were standardized across treatments and no supplemental irrigation was applied. Data collected included fruit yield, pH, ºBrix, and titratable acidity (TA). Data were analyzed with SAS PROC GLIMMIX; differences in means were determined at $\alpha < 0.05$. Plots supplemented with *B. mycoides* had lower fruit pH than untreated plots but higher fruit pH than the traditional fungicide program. Treatment regime did not influence Brix, TA, or total yield; however, all treated plots yielded more high quality fruit than the untreated control.

INTRODUCTION AND LITERATURE REVIEW

The grape (*Vitis sp.)* is one of the earliest domesticated fruit crops. The cultivation of grapes, known as viticulture, is important in several different cultures today and is deeply interconnected with that of winemaking. Grapes have several uses, including juices, fresh fruit, and raisins, but most often are fermented into wine (Pearson, 2009).

Early cultivation and domestication of the grapevine is believed to have occurred between the seventh and fourth millennia BC, in a geographical area between the Black Sea and Iran (Châtaignier, 1995; McGovern and Rudolph, 1996; Zohary, 1995; Zohary and Hopf, 2000). From this area cultivated forms of grapes were spread by humans to the Near East, Middle East and Central Europe. These areas acted as secondary points of domestication (Grassi et al., 2003; Arroyo-Garcia et al., 2006) from where viticulture gradually spread westward throughout Greece, Italy, and France (Laubenheimer and Brun, 2001). Indirect evidence of ancient winemaking is provided by the discovery of winemaking residues (tartaric acid) in clay jars, dating to the end of the seventh millennium BC (McGovern and Rudolph, 1996). The grape is now the most widely planted fruit crop in the world, covering an area of approximately 10 million ha ranging from temperate to tropical climates (Pearson, 2009). In the United States, there are approximately 410,000 hectares of land used for commercial viticulture (USDA, 2017).

Grapevines are a deciduous, woody, perennial vine. The growth pattern is characterized by a dormant season in the winter, followed by bud break in the spring. Early spring shoot growth precedes a vigorous growing season that slows by late summer as the vines begin to store carbohydrates, lose their leaves, and return to dormancy. Due to their vining growth habit, grapes are usually trained on a trellis system to allow them to be grown and pruned. Training and pruning is an important aspect of viticulture and helps to regulate vegetative growth and determine fruit load. There are several styles of trellis that can be utilized, with the common goal of managing the canopy and fruiting zone to optimize production and allow for ease of harvest. Canopy management in the vineyard is utilized to obtain a balance of air flow and sun exposure, aiding in the control of diseases, and exposing the fruit to adequate sunlight. Vines require 3 to 4 years for maturity which coincides with production level fruit yields. The first few growing seasons in a new vineyard are focused on vegetative growth. Grapes are propagated by cuttings of dormant canes, and are usually grafted in order to imbue resistance to phylloxera, a microscopic insect found in the soil that feeds on the roots of *Vitis.* Grafting is also utilized to control scion vigor and to increase lime tolerance, which is critical for many European growers (Pearson, 2009). One of the main difficulties during the cultivation of grapes for winemaking is the control of fungal diseases. Grapes are vulnerable to a wide range of fungal pathogens, including Botrytis bunch rot (*Botrytis cinerea),* powdery mildew (*Uncinula necator*), downy mildew (*Plasmopara viticola*), black rot (*Guignardia bidwellii*), phomopsis cane and leaf spot (*Phomopsis viticola*), anthracnose (*Elsinoë ampelina*), and several others (Anderson, 1956; Flaherty, et al., 1981)

Wild and cultivated grapevines are classified into the family Vitaceae. The genus *Vitis* contains 23 species, but only a few species are utilized commercially for production. The most important species produced are *V. vinifera, V. labrusca,* and interspecific hybrid crosses, known commonly as French hybrids. Known as the European grape, *V.*

vinifera vines are utilized primarily for winemaking. They are susceptible to all American pests and diseases, including phylloxera, downy mildew, powdery mildew, black rot, and Pierce's disease. Known as the fox grape or American grape, *V. labrusca* originated in the United States, and has a resistance to some fungal diseases such as downy and powdery mildew. Cultivated varieties such as 'Concord', 'Niagara', and 'Reliance' belong in this group and are popular in rainy regions because of their resistance to these fungal diseases. French hybrids were developed to utilize the disease and phylloxera resistance of the American varieties, while retaining much of the fruit quality and flavor profiles desirable in European wine grapes.

Grapes are best adapted to arid Mediterranean climates, which generally have low relative humidity and precipitation during the growing season. Grapes can now be found growing in all 50 states, and as a result are subject to a wide range of environmental conditions. Some environmental conditions such as those found in the southeastern United States are conducive to increased disease pressure by fungal pathogens. Most fungal diseases in grapes are favored by high relative humidity and free water, as well as the relatively low (10º to 30º C) temperatures found in this region. Currently, the most effective method to counteract their negative effects on grape quality and yield is the application of fungicides. While no one fungicide's active ingredient is effective on all pathogens, a variety of active ingredients are utilized in vineyard disease management programs. The majority of fungicides utilized in viticulture are effective by direct contact, which requires regularly scheduled (every 10-14 days) fungicide applications to protect new growth from pathogenic infection.

Although highly effective, fungicides with a very specific target site, or mode-of-action (MOA), are susceptible to resistance development by certain fungi. Overuse of one particular MOA can quickly lead to resistance. Quinone outside inhibitor class fungicides (QoI, Fungicide Resistance Action Committee (FRAC) group 11) are in this category. These fungicides are widely used in viticulture and can be highly effective on downy and powdery mildews, as well as black rot and anthracnose. However, a single point mutation in the cytochrome b gene confers resistance to QoIs in many plant pathogen species (Gisi et al., 2002). This resistance allows for a competitive advantage over wild populations of a pathogen, and encourages the resistant population to increase, when only one MOA is used. Studies have found that the competitive ability of resistant isolates relative to sensitive isolates varies depending on environmental conditions, including the initial frequency of resistant individuals in a population (Hagerty et al., 2017). A study in Kentucky (Gauthier and Amsden, 2014) found that a grower exceeded the maximum applications of 2 QoI fungicides between 2011-2012, resulting in 90% downy mildew incidence that did not respond to fungicides Abound 2.08F (azoxystrobin) and Pristine (pyraclostrobin + boscalid). A proper fungicide rotation utilizes different MOAs used in combination or alternation with high-risk fungicides such as QoIs, which help to reduce the chances for resistance development. (Rutgers Cooperative Extension, 2015) Understanding the effects of fungicide resistance is important to the development of resistance mitigation strategies. (Van Den Bosh et al., 2014)

According to Brent and Holloman (2000), there have been market driven concerns about fungicide residues and the need to manage fungicide resistance. As a result, alternative measures to protect crops (Crisp et al., 2006; Yildirim et al., 2002) have been studied for efficacy in controlling particular diseases such as powdery mildew and botrytis bunch rot. One experiment (Evans et al., 2012) utilized Aerated Compost Tea (ACT) applied to foliage and fruit of grapevines. ACT was assessed for its potential to suppress botrytis bunch rot and powdery mildew. Multiple applications of ACTs at two vineyards suppressed powdery mildew to <1% mean severity on Chardonnay leaves and bunches; compared to 77% severity for non-treated.

Another study evaluated chitosan**,** a substance derived from the shells of crustaceans for its ability to stimulate grapevine plant development and to induce protection from *B. cinerea* in *V. vinifera* plantlets. The study found that chitosan can be used in the vineyard as a means to attain protection from *B. cinerea*, and that its application may reduce the wide use of chemical pesticides (Barka et al., 2003). Previous research has found that chitosan and its derivatives are known to form a semi-permeable film around plant tissues, are inhibitory to a number of pathogenic fungi, and induce host-defense responses (El Ghaouth et al., 1997). There is increasing interest in biological control agents (BCAs) which utilize isolated strains of bacteria such as *Bacillus subtilis, B. amyloliquefaciens,* and *B. mycoides* to aid in the control of disease in the vineyard. Often, these BCAs can be effective on a wide range of crops, since they do not target one specific pathogen. A recent study found that although not fully effective alone, spray schedules based on integration of BCAs with fungicides are effective against *B. cinerea* and reduce the risk of fungicide resistance and fungicide residues in grapes (Rotolo et al., 2017).

Although not fully understood, Plants have a natural defense mechanism in response to exposure to pathogens, known as systemic acquired resistance (SAR). This defense response is induced following a localized exposure to pathogens, or certain biological and synthetic chemicals. SAR provides a relatively long-lasting period of resistance against unrelated pathogens such as viruses, bacteria, and fungi. (Ryals et al., 1994, 1996; Gozzo, 2003). Madamanchi and Kuc (1991) extensively studied the broad spectrum of SAR, and concluded that it was independent of the nature of the initial inoculant. The onset of SAR is closely associated with a local and systemic increase in endogenous salicylic acid (SA) (Metraux et al., 1990; Vlot et al., 2009) which has been proposed to be the signal for induced resistance. Recent studies on the mode-of-action of SA in inducing SAR revealed that SA itself is not the long-distance signal, although it is essential for the establishment of SAR (Vlot et al., 2009). SA accumulation triggers synthesis of pathogenesis-related (PR) proteins including NPR1 which induces defense gene expression, characterized by thickened cell walls (Hunt and Ryals, 1996; Van Loon and Van Strien, 1999). These PR proteins are produced by plants as a defense against pathogens and are known for their potential as biocontrol agents (Linthorst and Loon, 1991). One commercial synthetic chemical that induces SAR on a wide range of agricultural crops is sold under the trade name Actigard® (acibenzolar-S-methyl). This product belongs in a class known as benzothiadiazols (BTHs), which are not phytotoxic to crops (Gorlach, 1996). Actigard® is labeled for the control of several listed fungal, bacterial, and viral plant diseases.

An isolated form of *B. mycoides*, sold under the trade name LifeGard™ (*Bacillus mycoides* Isolate J, BmJ) has been shown to induce systemic resistance in a wide range of plants, and provide control for a range of diseases caused by fungi, bacteria, and viruses (Jacobsen, et al., 2004). Field experiments (Neher et al., 2009) evaluated applications of

BmJ and fungicides for the control of anthracnose in cucumber and cantaloupe. BmJ was compared to full and half labeled rate alternate applications of azoxystrobin and chlorothalonil. BmJ applied seven days before inoculation reduced disease severity by 41% in cucumber in 2004 and by 24% in cantaloupe in consecutive years compared to water controls, which was statistically equal to the fungicide treatments.

Although labelled for use on grapevines, there is limited research with *B. mycoides* isolate J on its efficacy in grapes grown in the southeastern United States. It is therefore the purpose of this study to measure the ability of *B. mycoides* Isolate J as a biological control agent (BCA) to elicit systemic acquired resistance (SAR) in a vineyard rotational fungicide program. Used in conjunction with traditional fungicides, this study measures the effect of reduced fungicide applications on yield, fruit chemistry, and overall fruit quality at the time of harvest.

MATERIALS AND METHODS

Field plots were established at the Agriculture Research and Education Complex in Bowling Green, KY in 2017 to investigate *Bacillus mycoides* isolate J (LifeGard™) as a supplement to a vineyard fungicide program for systemic acquired resistance (SAR) and its subsequent effects upon crop yield, fruit quality, and berry chemistry (titratable acidity, pH, °Brix). The experiment was conducted on 9-year-old *Vitis vinifera* L. 'Chambourcin' grapevines planted at a population of 1,350 vines ha^{-1} and trained on a vertically shoot positioned (VSP) trellis system. The experiment consisted of 3 replications of 4 treatments in a randomized complete block design (Table 1). Each plot consisted of 6 vines. The data were collected from the 4 center vines of each treatment to account for spray drift between treatments during fungicide applications. Fungicide treatments consisted of an untreated control, a program modelled from the Midwest Fruit Pest Management Guide 2017 (Traditional) and an identical program supplemented with 140 g ha^{-1} LifeGard™ per application (Traditional + LifeGard) which were both applied on 14 day intervals, and a reduced frequency application every 28 days supplemented with 140 g ha^{-1} LifeGard™(Reduced + LifeGard). Canopy management, fertility, herbicide and insect management were standardized across treatments and no supplemental irrigation was applied. A soil test prior to study establishment determined a pH of 7.1. An application of 316 kg ha^{-1} diammonium phosphate (DAP) and 30 kg ha^{-1} elemental sulfur was made to the research area on March 22 as recommended by the soil test report. A dormant season application of liquid lime sulfur (Sulforix™) was applied at 5 kg ai ha^{-1} to all vines used in the experiment on March 30. Initial treatment application began on April 25 when average shoot length was 14 cm. All treatments were applied with a SOLO 425DX backpack sprayer delivering 150 L ha^{-1} at 2 Bar. Subsequent treatments occurred every 14 days or 28 days, respectively. The reduced schedule received an initial 14-day reapplication during the pre-bloom stage, before reducing to its 28-day schedule. Products used and rates applied are listed in Table 2 and the treatment schedule in Table 3. All fruit was harvested September 29 from the center 4 vines of each treatment. Total yield was measured and the fruit was further separated into 3 grades based on quality. Grading was done visually for each cluster harvested. Grade 1 consisted of 0-33% cluster damage, Grade 2 consisted of 34-65% cluster damage, and Grade 3 consisted of 66-100% cluster damage (see Figure 1). Each grade was then weighed to determine graded yield for each treatment. Random samples of 4 clusters (approximately 200 berries per sample) were then collected from each treatment and sent to the lab for analysis. Samples were brought to 22° C, then crushed and strained through a mesh bag and the juice allowed to settle for 15 minutes before analysis. For each treatment sample, 1 mL of juice was collected in a 5 mL sterile syringe and 2 drops were used to measure ºBrix with an auto temperature compensating hand refractometer (Westover Scientific, Mill Creek, Washington, USA). A 20 mL juice subsample was taken from each treatment sample and pH was determined with an *Ultra*Basic pH Meter (Denver Instrument Company, Arvada, Colorado, USA). A 5 mL juice subsample was added to 50ml deionized water, and a 50 mL burette was filled with 0.1 N sodium hydroxide (NaOH). The juice sample was stirred with a stir plate and magnetic stirrer while NaOH was titrated to bring the juice pH to 8.2. The volume of NaOH used to neutralize the juice was recorded and used to determine titratable acidity (TA). Data were analyzed using SAS 9.4 software (SAS/STAT, 2013). Normality was analyzed using Shapiro – Wilks test by

PROC UNIVARIATE. Homogeneity of variances was analyzed using Brown – Forsythe test by PROC GLM. Data were analyzed in a one-way ANOVA using PROC GLIMMIX and significance was determined at α= 0.05.

Table 1. Plot Diagram for Fungicide Treatments

Row 1	104	103	101	102
Row 2	203	201	202	204
Row 3	302	304	303	301

Fungicide Treatment	**Plots**
Untreated Control	101,201,301
Traditional	102,202,302
Traditional + LifeGard	103,203,303
Reduced + LifeGard	104,204,304

Table 2. Fungicide Application Schedule

	Untreated	**Traditional**	**LifeGard**	**Reduced + LifeGard**
Week 1	NA*	Manzate	Manzate + LifeGard	Manzate + LifeGard
Week 3	NA	Manzate	Manzate + LifeGard	Manzate + LifeGard
Week 5	NA	Manzate	Manzate + LifeGard	NA
Week 7	NA	Quintec + Abound	Quintec + Abound + LifeGard	Quintec + Abound + LifeGard
Week 9	NA	Revus + Quintec	Revus + Quintec + LifeGard	NA
Week 11	NA	Rally + Phostrol	Rally + Phostrol + LifeGard	Rally + Phostrol + LifeGard
Week 13	NA	Phostrol	Phostrol + LifeGard	NA
Week 15	NA	Revus + Quintec	Revus + Quintec + LifeGard	Revus + Quintec + LifeGard

*NA = not applicable

Table 3. Fungicide Active Ingredients (a.i.) and Rates Applied

Fungicide	**a.i. / (FRAC*)**	**Rate applied per hectare (a.i.)**
LifeGard™	*B. mycoides* (P06)	121 g
Manzate™	mancozeb (M03)	2.6 kg
Rally™	myclobutanil (3)	112 g
Abound™	azoxystrobin (11)	219 g
Revus™	mandipropamid (40)	145 g
Quintec™	quinoxyfen (13)	55 g
Phostrol™	phosphorus acid (33)	2.3 kg

*FRAC = Fungicide Resistance Action Committee code

Grade 1 (0-33% damage)

Grade 2 (34-66% damage)

Grade 3 (67-100% damage)

Figure 1. Visual Grading for Fruit Quality

RESULTS

I. Yield

Total yields were not influenced by treatment as shown in Figure 2. Although not statistically different, there was an 11% greater yield in the full schedule *B. mycoides* supplemented treatment as compared to the reduced frequency supplemented treatment and the traditional treatment. Furthermore, the traditional and reduced frequency treatments had similar yields of 16.6 tonnes ha^{-1}. Visual grading indicated increased fruit quality for all treated plots when compared with the untreated control (Figures 3,4,5). Untreated plots had lower grade 1 fruit and higher grade 3 fruit (P<0.05).

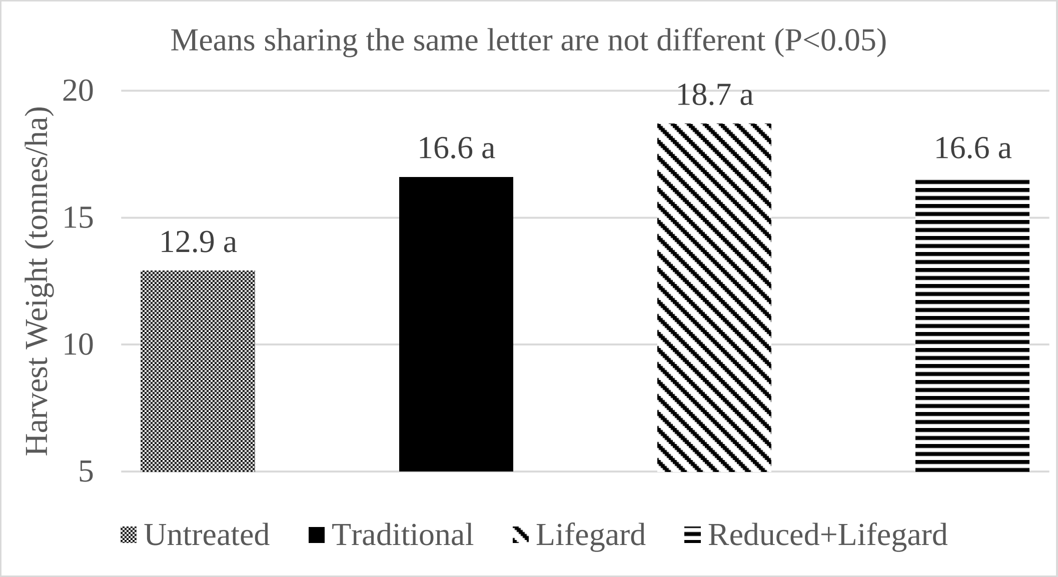

Figure 2. Total Yield as Influenced by Treatment

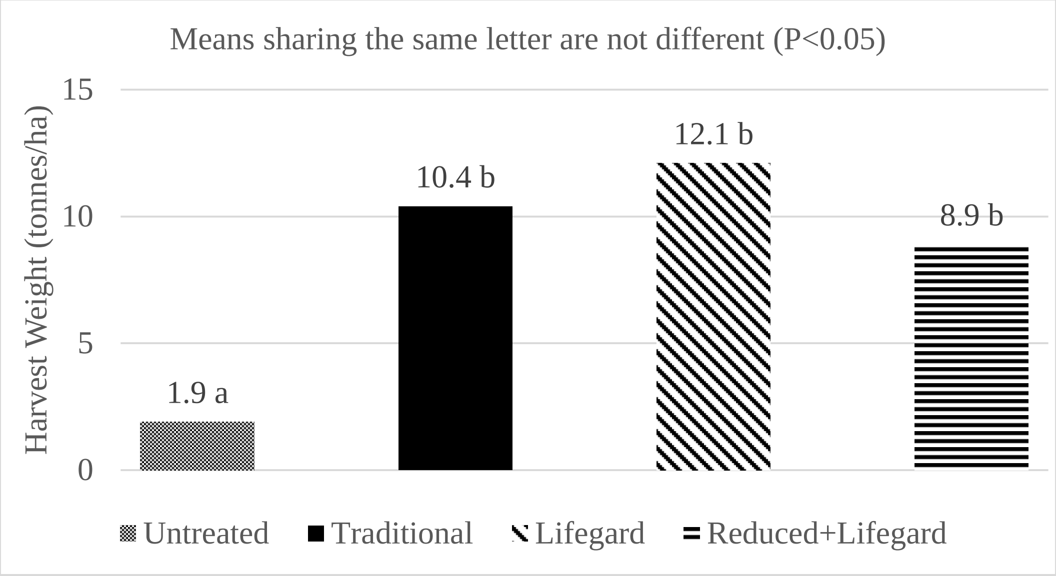

Figure 3. Grade 1 Yield as Influenced by Treatment

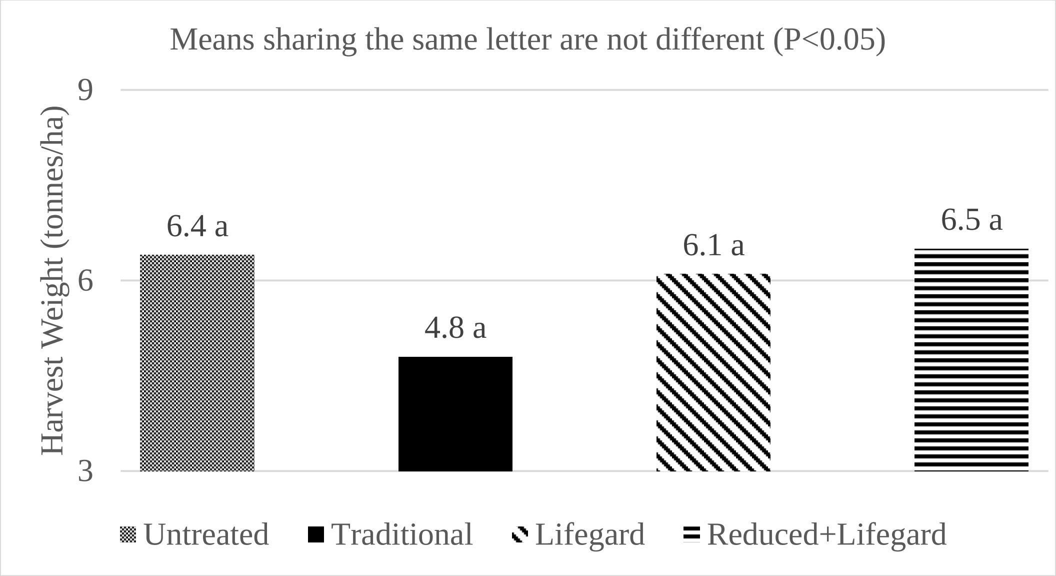

Figure 4. Grade 2 Yield as Influenced by Treatment

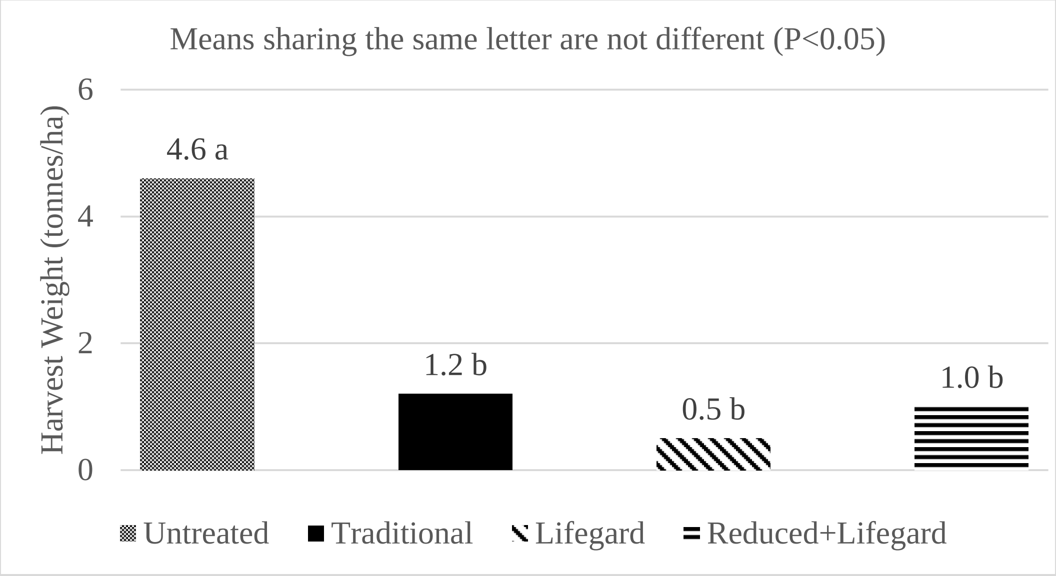

Figure 5. Grade 3 Yield as Influenced by Treatment

II. Fruit pH

Fruit pH was influenced ($P<0.05$) by treatment as shown in Figure 6. Plots supplemented with *B. mycoides* had lower fruit pH than untreated and were not different from each other. Reduction in application frequency did not affect pH when compared with full schedule LifeGard™ supplemented application.

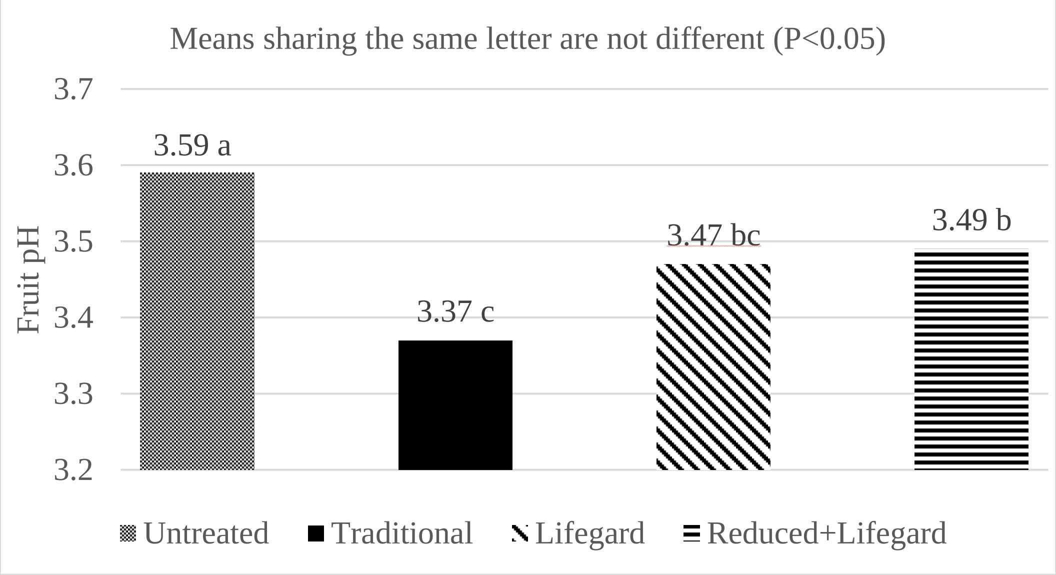

Figure 6. Fruit pH as Influenced by Treatment

III. °Brix

Treatment did not influence °Brix as shown in Figure 7. No differences were observed.

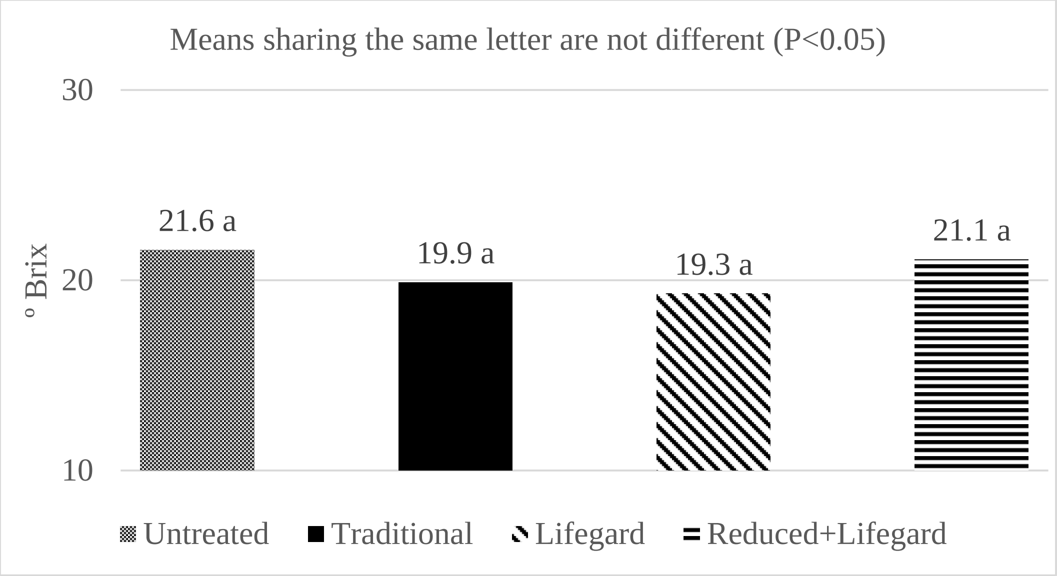

Figure 7. Fruit °Brix as Influenced by Treatment

IV. Titratable Acidity

Treatment did not influence TA as shown in Figure 8. No differences were observed.

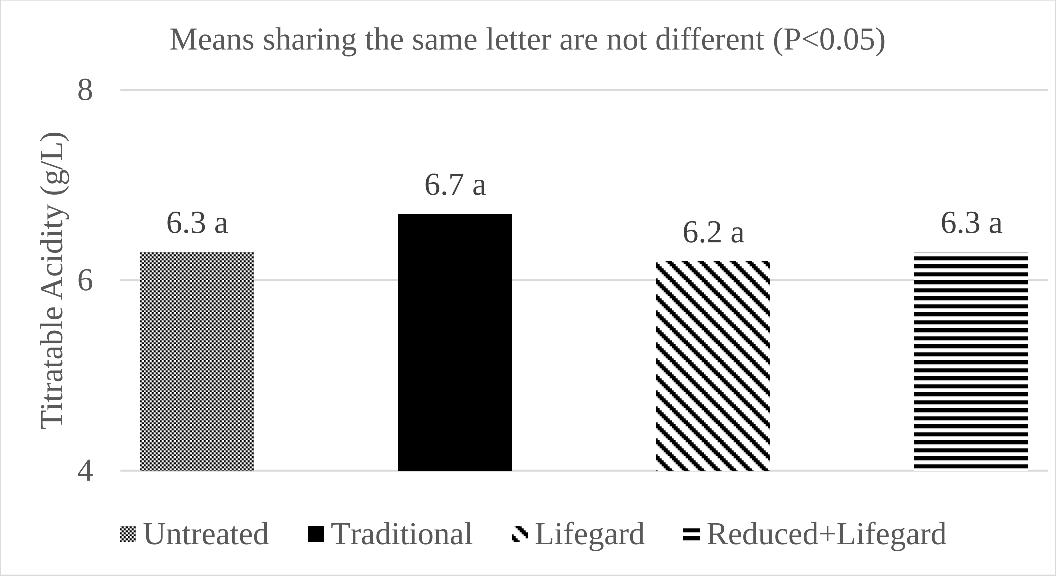

Figure 8. Titratable Acidity as Influenced by Treatment

DISCUSSION

This experiment examined the effect of *Bacillus mycoides* isolate J when used in combination with fungicides in a vineyard spray program. Grape producers are concerned with not only the yield potential of grapevines, but also the quality of the fruit harvested from those vines. As a result, measures are taken to protect crops from diseases which can have an adverse effect on yield and fruit quality. Infection of berries in the field can lead to increased levels of infestation by spoilage microorganisms, which substantially degrade wine quality. One example, the causal agent of powdery mildew, *Uncinula necator*, is one of the most destructive pathogens on grapevines. It colonizes leaves, the rachis, and fruit of the vine. This reduces yield and wine quality substantially, and can impart a very foul flavor to the wine (Ficke et al., 2002).

The results of this experiment indicate a correlation between the quality of fruit and the amount of disease symptoms observed in the field, as was expected. Untreated plots had significantly lower quality than treated plots. Low quality fruit is undesirable to both the producer and the consumer, and the results of this study show that an average increase of 82% grade 1 fruit occurred in the treated plots when compared with the untreated control ($P<0.05$). No significant total yield differences were observed between treatments. There was no significant difference in quality or total yield among any of the treated plots, however the numerically greatest total and grade 1 yields were observed in the traditional spray program supplemented with LifeGard™. Supplementing LifeGard™ into the traditional spray program resulted in a 14% increase in total yield, and a 21% increase in grade 1 fruit. This suggests that the additional mode-of-action (MOA) did have a positive impact on yield although it was not significant in this study. The reduced frequency program supplemented with LifeGard™ resulted in statistically similar yields with other treated plots, which suggests that supplementing LifeGard™ may be a viable method to reduce fungicide application frequency while simultaneously providing an additional MOA to help combat resistant strains of fungi.

Fruit chemistry is an important aspect of grape juice and winemaking. It is measured both in the vineyard and during the winemaking process. The °Brix correlates to the sugar content of the grape, and as a result the potential alcohol of the finished wine. Acid content is measured as pH and titratable acidity (TA). TA in wine is applied to sensory perception of a wine’s acidity, i.e. tartness or crispness, while pH is a measurement of the likelihood and speed of occurrence of pH dependent reactions. (Boulton, 1980). The pH level has an impact on color, microbial stability, and the amount of sulfur dioxide (SO_2) required to protect wines from oxidation and spoilage. Grapes contain 2 primary acids, tartaric and malic. In general, as berries ripen, malic acid content will decrease and sugar content will increase, while the tartaric acid level remains relatively unchanged. As ripening occurs, sodium and potassium ions are transported to berries, resulting in the formation of large amounts of acid salts. The buffering action of the acid salts, combined with the loss of malic acid, results in a noticeable rise in juice pH (Fowles, 1992). Grape harvest is determined when both sugar and acid content fall within an acceptable range and will vary from season to season. It has been reported that French-American hybrids in the eastern U.S. are best at 19-23 °Brix and that high quality red grapes have a pH around 3.4, and a TA of 7.5 g L^{-1} (Cox, 2015). Determining when to harvest can vary from grower to grower, and also at the preference of the consumer.

Treatment had no effect on °Brix or TA, and all of the sample means fell within the acceptable range for harvest. The pH was significantly affected by treatment, with the untreated control having the highest pH of 3.6. This is likely due to the substantial disease pressure which accelerated ripening and caused damage to berries, opening them to spoilage organisms which are known to change the chemical composition of juice depending upon the organism (Nelson and Ough, 1966). A 3.6 pH is within the acceptable range but combined with poor fruit quality would make the fruit highly undesirable for winemaking and susceptible to spoilage during fermentation. The pH of both LifeGard™ supplemented plots were within the highly desirable range for making red wines, and would be ideal for a fermentation that did not require a pH adjustment. Replication of these results using LifeGard™ could possibly provide a solution to growers in the region who experience high pH and high TA fruit at harvest. The combination of high pH and TA is a problem for winemakers because the wine already has a tart flavor, and the addition of tartaric acid commonly used to lower pH increases that perception and can cause an imbalance in flavor profiles. Wine fermentation that starts with a high pH is much more prone to spoilage organisms or failure during fermentation. Fruit that already has desirable berry chemistry requires little if any adjustment to pH or TA.

CONCLUSION

This experiment examined supplementation of *Bacillus mycoides* isolate J into 2 different fungicide application frequencies and the subsequent effect on yield, fruit chemistry, and overall fruit quality at the time of harvest. Fungicide treatments had a significant effect on fruit quality when compared with the untreated control. The additional mode-of-action provided by BmJ correlated to a numerical increase in total yield and fruit quality. A reduced fungicide application frequency supplemented with BmJ yielded similar results as demonstrated with the traditional fungicide applications, suggesting an overall reduction in fungicides may be possible with the supplementation of BmJ. A significant pH difference was observed in plots treated with BmJ. The pH and fruit chemistry of BmJ treated plots were desirable to winemakers and a replication of this experiment could provide a means of pH manipulation in the vineyard, with further research. Future research with BmJ should compare reduced fungicide applications with and without the supplementation of BmJ to determine the overall influence of BmJ in a reduced frequency fungicide program.

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