POTENTIAL USE OF BIOFUNGICIDES AND CONVENTIONAL FUNGICIDE FOR THE MANAGEMENT OF BOTRYTIS BLOSSOM BLIGHT IN LOWBUSH BLUEBERRIES

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ABSTRACT

Botrytis blight is an economically important disease of lowbush blueberry that causes significant yield loss annually. In this study, four biofungicides, Diplomat 5SC® (polyoxin D), Timorex Gold® (tea tree oil), Fracture® (BLAD) and Serenade MAX® (*Bacillus subtilis*) were evaluated for their disease suppression potential against *B. cinerea* individually and in rotation with Switch® under field conditions. Three applications of each biofungicide were made for the stand alone treatment at 7-10 days' interval with Switch® replacing the 2nd application in the combined treatment. Results indicated that the products have potential for use as a biofungicide in lowbush blueberries. All the stand alone and rotational applications brought about significant reduction in disease development, especially in 2019. The application of Diplomat 5SC® and Fracture®-Switch® rotation decreased disease development by over 63% in 2018. In 2019, all stand alone treatments reduced disease development by more than 42% whereas their rotation with switch reduced disease by over 69% at Earltown and at least 30% at Farmington. Stand alone Diplomat 5SC® and Timorex Gold® along with Fracture®, Timorex Gold® and Serenade MAX® rotation resulted in over 20% more berries. This study suggests that the biofungicides and their integration with chemical fungicides have the potential as an alternative management strategy against Botrytis blossom blight to reduce the use of conventional fungicides and produce fruit with no detectable fungicide residues.

Keywords: Biofungicides, blueberry, *Bacillus subtilis*, BLAD, polyoxin, tee tree oil, *Vaccinium angustifolium*

INTRODUCTION

The Lowbush blueberry (wild blueberry) (*Vaccinium angustifolium* Ait. and *V. myrtilloides* Michx.) is native to North America and fields are developed from forested areas or abandoned farmland through the removal of compiting vegetation. Lowbush blueberries are an important crop and a leading horticultural commodity in Atlantic Canada. The crop is produced on approximately 40,500 ha and accounts for ~30% of Canada's land area in fruit, berries and nut production (Statistics Canada, 2019a). In 2018, the Atlantic Canadian provinces and Quebec produced approximately 57.3 million kg of wild blueberries with farm gate value ~ $60.9 million (Statistics Canada, 2019b).

Lowbush blueberry plants can encounter several fungal disease challenges. Historically, Botrytis blossom blight has been a major problem in wild blueberry production, especially in coastal areas with prolonged wet conditions. The disease is caused by the pathogen *Botrytis cinerea* Pers.: Fr. that typically infects flowers or entire inflorescences at the mid to late bloom stage (Hildebrand et al., 2001). The disease causes over 20% yield loss annually and over the past decade, it has become far more prevalent due to increased canopy densities, longer wetness durations and more susceptible floral tissue (increase in flower densities from 93 million flowers ha^{-1} in 1994 to over 370 million flowers ha^{-1} due to improved practices such as nutrient and weed management) (Percival, 2013).

Given that Botrytis blight is caused by a fungus classified as posing high risk of developing fungicide resistance (FRAC, 2019), fungicides with different modes of actions are used in controlling the disease (FRAC, 2010). The main fungicide presently used for Botrytis blight control in wild blueberry is Switch®, which contains the signal transduction and amino acid inhibitors fludioxonil and cyprodinil, respectively. Other fungicides used include Luna Tranquility®, Fontelis® and Pristine® (Burgess, 2018, Percival, 2013). Although there are several fungicides, the management of the pathogen is challenging due to its high-risk nature, fungicide cost, concerns about fungicide residue and strict maximum residue limits (MRL) allowed on the international market. With Botrytis management products accounting for more than 60% of the fungicide costs and concerns over detectable residues by consumers, there is a need to develop disease management approaches that will deliver no detectable levels of fungicide residues in processed fruit. This has given rise to the interest in biofungicides including Serifel™ (*Bacillus amyloliquefaciens*), Serenade Max® (*Bacillus subtilis*) and Fracture™ (BLAD polypeptide) (Percival et al, 2016). Previous studies have reported that some biological control agents, plant extracts and biologically active natural products can serve as excellent alternative to conventional fungicides presently being used (Shao et al., 2013; Monteiro et al., 2015; Li et al., 2017; Jiang et al., 2018; Abbey et al., 2019; Calvo-Garrido et al., 2019). Some essential oils like the tee tree oil from *Melaleuca alternifolia* have been reported to be effective against plant pathogens including *B. cinerea* (Nguyen et al., 2013; Potočnik et al., 2010). Similarly, polyoxin extracted from the soil bacterium (*Streptomyces cacaoi* var.*asoensis*) (Mamiev et al., 2013), has been reported to effectively supress *B. cinerea* in strawberry (Dowling et al., 2016; Nguyen et al., 2013).

A number of bio-fungicides have recently been developed, but their efficacy against Botrytis blossom blight in lowbush blueberry production systems have not been evaluated. While adequate blight control has not been obtained when the products are used alone, preliminary studies have demonstrated that adequate disease suppression can be achieved when biofungicides are combined with the conventional fungicide by way of rotation (Percival et al, 2016, Abbey et al., 2020) and/or when conventional fungicides are used during peak disease pressures. In view of the continuous search for more economically and environmentally friendly alternative for conventional fungicides, it is important to evaluate new products that have been shown to suppress *Botrytis cinerea* in other crops. The objective of this research was to determine the efficacy of commercially formulated tea tree essential oil, polyoxin D, BLAD and *Bacillus subtilis* used alone and in combination with conventional fungicide for the control of Botrytis blossom blight in wild blueberry fields.

MATERIALS AND METHODS

Site selection and experimental design

Field trials using biofungicide treatments against Botrytis blossom blight were carried out in two consecutive years at two different locations in each year. In 2018, experiments were conducted at Pigeon Hill (coordinates = 45°34′35.03 N, 63°51′54.84 W) and Blue Mountain, NS (coordinates = 45°28′53.29 N, 62°25′27.26 W), and at Farmington (coordinates = 45°34′24.20 N, 63°53′37.84 W) and Earltown, NS (coordinates = 45°34′50.58 N, 63°06′05.15 W) in 2019. A randomized complete block design (RCBD) with five replications was used. Plot size was 4 × 6 m with 2 m buffers between plots. Fields for the experiments were equipped with Watchdog® model 2700 weather station (Aurora, IL, USA) to monitor air temperature, relative humidity, leaf wetness, wind speed and direction every 15 min for the duration of the trial.

Fungicide products and treatment application

Ten treatments were included: (1) untreated control; (2) Diplomat 5SC®; (3) Timorex Gold®; (4) Fracture®; (5) Serenade MAX®; (6) Diplomat 5SC® - Switch® - Diplomat 5SC®; (DSD) (7) Timorex Gold® - Switch® - Timorex Gold® (TST); (8) Fracture® - Switch® - Fracture® (FSF); (9)

Serenade MAX® - Switch® - Serenade MAX® (SSS); and (10) Fontelis® - Switch® - Pristine® (LSP). The active ingredients and the application rates of products are indicated in Table 1.

Fungicide application

First fungicide application was made at 10% bloom stage prior to visual symptoms of Botrytis blight. The second application was made 7 to 10 days after the first application, and the third application was made 14 to 17 days after the first application. Fungicides were applied using a hand-held CO_2 research sprayer (Bell spray Inc.) with a 2 m boom equipped with 4 Tee Jet Visiflow 8003VS nozzles at a pressure of 32 psi.

Disease assessment, yield component, berry yield and statistical analysis

Fifteen blueberry stems were randomly selected seven days after the second fungicide application and 14–17 days after the third fungicide application for disease assessment. The stems were cut diagonally at 20 cm intervals along a 4 m line transect in each plot. The stem samples were placed in plastic bags and taken back to the laboratory for assessment of Botrytis disease development (incidence and severity). Disease incidence was determined as the proportion of floral buds with visual symptoms of Botrytis blight within a stem expressed as a percentage. Disease severity was assessed as the percentage of floral tissue area infected with visual symptoms of Botrytis blight on a stem. A 0–7 disease severity rating scale was used where 0 = no symptoms, healthy plants; 1 = 0–5% affected flower area; 2 = 5–15% affected flower area: 3 = 15–35% affected flower area; 4 = 35–65% affected flower area; 5 = 65–85% affected flower area; 6 = 85–95%; 7 = 95–100% affected flower area. The data were expressed as a percentage of affected flower area (disease severity).

Yield components (number of fruit set per stem) were measured in early August by randomly selecting 15 stems per plot. Berries were harvested in August with a lowbush blueberry hand rake from four randomly selected 1 m^2 quadrants in each plot. Harvested berries from each plot were weighed with an Avery Mettler PE 6000 digital balance, and the data was recorded.

Data collected on disease development and harvested berries were checked for normality prior to analysis and harvested berries were square root [√ (×)] transformed to ensure normality. All the data were analyzed using the PROC GLIMMIX procedure of SAS (version 9.4, SAS institute, Inc., Cary, NC). Least Significance Differences (LSD) was used for multiple means comparisons at $\alpha=0.05$.

RESULTS

Botrytis blight disease pressure was low in the two trials in 2018 with 1.3 and 10.7 % of the total stems assessed (n=750) showing Botrytis blight symptoms and signs at Blue Mountain and Pigeon Hill, respectively after second fungicide application. After the third fungicide application, 0.35 and 1.1 % of the total stems assessed showed Botrytis blight at Blue Mountain and Pigeon Hill, respectively (Table 2). Contrary to 2018, high disease pressures were observed in 2019 with 30.4 and 32.2 % of assessed stems showing disease symptoms at Earltown and Farmington, respectively after the 2nd application. After the 3rd fungicide application, 9.20 and 26.3 % of assessed stems showed disease symptoms at Earltown and Farmington, respectively (Table 3).

In 2018, disease incidence and severity ranged from 1.65 to 11.7% and 1.65 to 11.6%, respectively, after the 2nd application, and 0 to 1.81 for both incidence and severity after the third application at Pigeon Hill (**Table 2**). After the 2nd fungicide applications, there was significant treatment effect on disease development. The application of Diplomat 5SC and FSF significantly lowered incidence by 76 and 68 %, and severity by 69.4 and 63 %, respectively compared to the untreated control. On the contrary, there was no significant treatment effect on disease development after the third fungicide application. In the trial at Blue Mountain, there was no significant treatment effect ($P > 0.05$) on disease development after second and third fungicide applications probably due to the very low disease levels (**Table 2**).

In 2019, significant treatment effect was observed at Earltown with disease incidence and severity ranging from 6.90 to 41.9 % and 2.97 to 34.6 %, respectively after the second application. Incidence range of 1.10 to 16.7 % and severity of 0.33 to 13.0 % were observed after the third application at Earltown **(Table 3)**. After the second fungicide application, stand-alone Diplomat 5SC, Timorex Gold, Facture and Serenade Max significantly reduced disease incidence by 78.7, 43.3, 42.3 and 60.5%, respectively and severity by 83, 71.7, 46.7 and 71.2 %, respectively compared to the untreated control. The rotation of all the biofungicides with Switch (DSD, TST, FSF, SSS) and conventional control program (LSP) highly suppressed disease development with over 69 and 81 % less incidence and severity (**Table 3**). All the stand alone treatments reduced disease incidence by more than 50 % and severity by over 42 % after the 3rd fungicide application (**Table 3**). The rotation of all the biofungicides with Switch® significantly reduced disease incidence and severity by more than 78 and 77 % compared to the untreated control. Interestingly, disease suppression provided by Diplomat 5SC, DSD, TST, FSF and SSS were comparable to that of the LSP **(Table 3)**. At Farmington, disease incidence and severity ranged from 7.59 to 32.9 % and 3.41 to 23.2 %, respectively after the second application. After the 3rd fungicide application, incidence and severity ranged from 4.09 to 23.8 % and 1.92 to 10.8 %, respectively (**Table 3)**. After the second fungicide application, both stand-alone and their rotation with Switch® significantly reduced disease with over 55 and 66 % less incidence and severity, respectively. Disease control achieved by stand-alone treatments and their rotation with Switch® were comparable to the disease suppression achieved by LSP. After the third fungicide application, Diplomat 5SC, TST and FSF significantly reduced disease incidence and severity by over 30 and 37 %, respectively which were comparable to the LSP **(Table 3)**.

There was a significant treatment effect on yield components at Pigeon Hill and Blue Mountain in 2018. At Pigeon Hill, Fracture, TST, and FSF resulted in significantly high set fruit per stem with over 30% more et fruit than untreated control and LSP (Table 5). At Blue Mountain, the convention control program resulted in the highest number of set fruit (5.04) followed by Serenade Max. Diplomat 5SC, Timorex Gold, TST, and FSF also resulted in high set fruit which were comparable to the convention control program.

In 2019, there was no significant treatment effect (P > 0.05) on yield components at Earltown. At Farmington, Diplomat 5SC, Fracture, FSF, SSS and LSP resulted in higher set fruit per stem with over 28% more set fruit compared to the untreated control (Table 5).There was a significant treatment effect on harvestable berry yield at Pigeon Hill and Blue Mountain in 2018. Diplomat 5SC, TST, FSF and SSS resulted in improved berry yield compared to the untreated control with over 20.5, 34.7, 26.0 and 33.2% more berry yield, respectively (Table 6). Diplomat 5SC, Timorex Gold and FSF resulted in in improved berry yield 14, 8 and 7%, respectively.

In 2019, there was a significant treatment effect on berry yield. Although significant, most of the treatments were not different from the untreated control and among each other **(Table 6)**. All the stand-alone biofungicide applications resulted in over 20 % yield increase compared to the untreated control. Similarly, FSF and SSS increased yield with 17 and 22.7 %, respectively **(Table 6).**

DISCUSSION

Botrytis cinerea is a high-risk polycyclic pathogen which causes floral blight disease in blueberries under conducive environmental conditions. In view of this, frequent application of control products, especially chemical fungicides are carried out to maintain high crop value and reduce yield losses in fields. In spite of this, *B. cinerea* continues to cause significant losses in lowbush blueberry fields due to the development of resistance among the pathogen population (Abbey 2017). To this effect, the implementation of an integrated disease management strategy that involves the use of both biofungicides and conventional fungicides is essential for the successful control of Botrytis blossom blight in lowbush blueberry fields.

In this study, the application of biofungicides were able to suppress Botrytis blight infection in both years, however, disease pressures varied between the two years and the time of disease assessment. Although there were significant Botrytis infection periods throughout the flowering period in at both years, environmental conditions played a significant role in this variation in disease pressures (supplementary material, Figures S1-S4, Tables S1 – S4). A significant frost occurrence (-3.3 ^{0}C, -2.5) (supplemental material, Figure S-1 and S-2) affected the flower tissues which explains the low disease pressures in 2018 compared to 2019. Also, flower tissues assessed after the second fungicides application had higher disease because the second application occurred at full bloom (stages F6 – F7), a stage at which flower tissues are most susceptible to disease infection (Hildebrand et al., 2001; Abbey et al., 2018).

This study demonstrated that, the application of biofungicides significantly reduced disease development. Generally, many natural compounds including polyoxin D (Dowling et al., 2016; Brannen et al., 2020;), banda de lupinus albus doce (BLAD) (Monteiro et al., 2015; Abbey et al., 2020) and tea tree oil (Cheng and Shao, 2011; Shao et al. 2013) as well as biocontrol agents such as *Bacillus* spp. (Lee et al., 2006; Martínez-Absalón et al., 2014) are well known to be effective in the management of *B. cinerea* and several plant pathogens. Due to the extensive research in the use of biofungicide, several modes of action are known to exist and are well understood. While some are known to have simple and direct mode of actions, other such as *Bacillus* spp. are known for their complex modes of action (Cawoy et al. 2011). Polyoxin D has been reported to interfere with the activities of chitin synthetase enzyme which results in the inhibition of chitin formation in the fungal cell wall (Becker et al., 1983; Adaskaveg et al., 2011). Similarly, BLAD is also known to interfere with fungal chitin by binding and degrading chitin through the removal of the N-acetyl-D-glucosamine terminal in chitin (Monteiro et al., 2015; APVMA, 2017). Also, the terpenoids (terpinen-4-ol) content of tea tree oil has been reported to act on cell membranes and alter the permeability of fungal cells including *B. cinerea* (Carson et al., 2006; Yu et al., 2015). *Bacillus* spp, has been identified to suppress pathogen through the production of antibiotics and induction of host resistance (Niu et al., 2011; Pathma et al., 2011; Chowdappa et al., 2013; Ji et al., 2013). Given the extensive reports on the use of biofungcides and their modes of action, it is not surprising that Diplomat 5SC ®, Timorex Gold®, Fracture® and Serenade MAX® suppressed Botrytis blossom blight in lowbush blueberry, and in some cases were comparable to the convention control program in this study.

Generally, the combination of different biofungicides have been touted to be an effective way of disease control. The combination of different biofungicides helps address the inconsistencies in disease control experienced with stand alone application of biofungicides. The effectiveness of biofungicides are well known to be greatly influenced by environmental conditions such as temperature (Xu et al., 2011; De Cal et al., 2012; Sylla et al., 2015). As a part of addressing this challenge, many studies have achieved significant disease control when biofungicides are combined with chemical fungicides (Gilardi et al., 2008; Boukaew et al., 2013). In this study, it was hypothesis that the integration of biofungicide with a chemical fungicide has the potential to improve efficacy and reduce variability of biofungicides often experied in the field conditions. It is therefore not surprising that the rotation of Diplomat 5SC ®, Timorex Gold®, Fracture® and

Serenade MAX® with Switch® resulted in significant disease control compared to the stand alone treatments, especially at the Earltown test field in this study (Table 3). It is also noteworthy that biofungicides and their rotation with the chemical fungicides provided disease control similar to the convention control program of three chemical fungicide applications. This is important because harvested fruit from fields treated with biofungiside or their rotation with a conventional are far less likely to have chemical residues.

The combination of biofungicide and Switch® (a.i fludioxonil (FRAC group 12), a signal transduction inhibitor and cyprodinil (FRAC group 9), an amino acid and protein synthesis inhibitor) with different modes of action falls in line with the concept of integrated pest management. This approach protects the various components of the management strategy from total failure. For instance, the application of Switch® will help prevent total disease control failure in the event that the environmental conditions do not favour the establishment of biocontrol agents or biodegradation of natural compounds. In addition, the rotation of biofungicide with Switch® helps to reduce the amount of chemical fungicide application from 3 to 1. This will have a practical implication on growers as they will have to use less amount of relatively expensive chemical fungicides whiles reducing environmental pollution from excessive use of chemical fungicides. Also, the timing of the Switch® application in these trials was based on the desire to ensure good disease control and avoid or completely reduce fungicide residue in harvested berries. This is possible because the chemical fungicide was applied at full bloom when disease pressures were most likely to be high (Hildebrand et al., 2001). It has been reported that biofungicides are less effective when disease pressures are high (Hofstein and Chapple, 1999; Reiss and Jørgensen, 2017), hence rotating the chemical fungicides is critical in achieving adequate disease control. The timing of Switch® treatment helps to extend the pre-harvest interval which can contribute significantly to residue reduction. Also the Switch® application was made while the flowers are in bloom and not as much of the developing ovary is exposed to the chemical fungicides. Being able to produce residue-free berries to meet international MRLs is very important, as the majority of lowbush blueberries are exported to Europe and Asia with strict and limited MRLs.

Although the biofungicides used in this study have shown great potential as alternative for conventional fungicides, there are some reports of resistance development among *B. cinerea* population from strawberry fields. For instance, *B. cinerea* isolates with reduced sensitivity to polyoxin have been reported from commercial strawberry fields in the USA (Dowling et al., 2016) and sweet basil in Israel (Mamiev et al., 2013). This could be because these natural compounds act directly on the fungi, and given the genetic diversity and high risk nature of *B. cinerea,* there is the potential for resistance development (Dowling et al., 2016). In view of this, resistance management may be necessary for control strategies which include polyoxin D.

In this study, the application of biofungicides and their rotation led to improved set fruit (fruit per stem). This can be attributed partly to the effective disease suppression obtained from the application of biofungicides. Although not all the fungicides resulted in consistent berry yield, it is worth mentioning that treatments such as Diplomat 5SC®, Timorex Gold®, TST, FSF and SSS resulted in ~20% more berries. The inconsistency observed among some of the treatments, can partly be attributed to the variability in plant populations in lowbush blueberry fields. Lowbush blueberries are native, and naturally occurring, hence significant variations exist among phenotypes and plants density from field to field (Hepler and Yarborough, 1991).

In conclusion, the outcome of this study provides information on the potential of introducing biofungicides into Botrytis blight management programs. The application of biofungicides alone and their rotation with Switch® yielded a promising result. Significant disease suppression was obtained with stand-alone application of biofungicide as well as their rotation with Switch®. This study revealed that biofungicides can stand-alone in the control of Botrytis blight, however, their integration with low risk fungicides is a more promising approach.

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Tables

Table 1. Product application rates and active ingredients of fungicides used for Botrytis blossom blight control.

Products	Active ingredients	Product application rates
Diplomat 5SC ®	Polyoxin D zinc salt	926 ml/ha
Timorex Gold®	Tea Tree Oil	1500 ml/ha
Fracture®	Banda de Lupinus albus doce, BLAD	2.6 L/ha
Serenade MAX®	*Bacillus subtilis* strain QST 713	6 kg/ha
Switch®	Cyprodinil and fludioxonil	975 g /ha
Fontelis®	Penthiopyrad	1.2 L/ha
Luna trainquility	Fluopyram and pyrimethanil	1500 g/ha

Table 2. Incidence and severity of Botrytis blight observed from Pigeon Hill, Nova Scotia after fungicide applications in 2018.

	2nd application		***3rd application***	
Treatment	**Incidence (%)**	**Severity (%)**	**Incidence (%)**	**Severity (%)**
Control	6.90 abc	5.40 bc	1.81	1.81
Diplomat 5SC	1.65 c	1.65 c	0.59	0.59
Timorex Gold	9.12 ab	9.18 ab	0	0
Facture	5.81 bc	5.54 bc	0	0
Serenade Max	5.72 bc	5.27 bc	0.36	0.36
DSD	5.87 bc	5.14 bc	0.79	0.64
TST	11.7 a	11.7 a	0	0
FSF	2.21 c	1.98 c	0	0
SSS	5.02 bc	3.44 c	0	0

LSP	5.71bc	5.71 c	0	0
ANOVA ^{z}	**p=0.0337**	**p=**0.0102	**NS**	**NS**

^{z} Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at p<0.05. Mean separation was completed using LSD test procedure. Data in a column with the same letters are not significantly different at ά=0.05.

Table 3. Incidence and severity of *Botrytis* blight observed from Earltown, Nova Scotia after fungicide application in 2019.

	2nd application		***3rd application***	
Treatment	**Incidence (%)**	**Severity (%)**	**Incidence (%)**	**Severity (%)**
Control	41.9 a	34.6 a	16.7 a	13.01 a
Diplomat 5SC	8.95 c	5.87 c	1.67 bc	1.00 cd
Timorex Gold	23.8 b	9.79 bc	7.57 b	4.58 bcd
Facture	24.2 b	18.4 b	8.32 b	7.50 b
Serenade Max	16.6 bc	9.97 bc	8.01 b	5.55 bc
DSD	6.90 c	3.67 c	2.27 bc	2.06 cd
TST	8.95 c	5.12 c	3.66 bc	2.93 bcd
FSF	11.1 c	6.37 c	1.11 c	0.94 cd
SSS	12.9 bc	3.99 c	2.79 bc	0.97 cd
LSP	8.13 c	2.97 c	1.10 c	0.33 d
ANOVA ^{z}	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p<0.0001**

^{z} Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at p<0.05. Mean separation was completed using LSD test procedure. Data in a column with the same letters are not significantly different at ά=0.05.

Table 4. Incidence and severity of Botrytis blight observed from Blue Mountain, Nova Scotia after fungicides application. Plant in 2018.

	2nd application		***3rd application***	
Treatment	**Incidence (%)**	**Severity (%)**	**Incidence (%)**	**Severity (%)**
Control	1.82	1.68	0	0
Diplomat 5SC	1.25	0.87	0	0
Timorex Gold	0.22	0	0	0
Facture	0	0	0	0
Serenade Max	0	0	0	0
DSD	2.67	0.02	0	0
TST	0	0	0	0
FSF	0.14	0.08	1.74	1.63
SSS	0	0	0	0
LSP	0	0	0	0

ANOVA [z]	**NS**	**NS**	**NS**	**NS**

[z] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at p<0.05. Mean separation was completed using LSD test procedure. Data in a column with the same letters are not significantly different at ά=0.05.

Table 5. Incidence and severity of Botrytis blight observed from Farmington, Nova Scotia at after fungicide application in 2019.

	2nd application		***3rd application***	
Treatment	**Incidence (%)**	**Severity (%)**	**Incidence (%)**	**Severity (%)**
Control	32.9 a	23.2 a	14.5 bc	7.37 ab
Diplomat 5SC	9.3 b	6.81 b	10.1 bcd	4.62 bcd
Timorex Gold	14.2 b	6.17 b	14.1 bc	8.11 ab
Facture	11.3 b	4.50 b	23.9 a	10.6 a
Serenade Max	10.2 b	4.69 b	14.9 bc	6.75 abc
DSD	11.5 b	4.33 b	16.9 ab	10.0 a
TST	11.6 b	7.84 b	8.23 dc	2.55 cd
FSF	14.7 b	4.89 b	16.9 ab	4.63 bcd
SSS	12.7 b	3.41 b	16.5 abc	9.40 a
LSP	7.59 b	3.41 b	4.09 d	1.92 d
	p=0.0008	**p<0.0001**	**p=0.0004**	**p=0.0002**

[z] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at p<0.05. Mean separation was completed using LSD test procedure. Data in a column with the same letters are not significantly different at ά=0.05.

Table 6. Yield component and harvestable berry yield observed at Pigeon Hill after biofungicide applications

	2018		**2019**	
	Pigeon Hill	**Blue Mountain**	**Earltownn**	**Farmington**
Treatment	**(g/m^2)**	**(g/m^2)**	**(g/m^2)**	**(g/m^2)**
Control	252.8 de	383.3 ab	252.3 a	566.7 c
Diplomat 5SC	317.9 abcd	437.2 a	118.9 f	779.0 ab
Timorex Gold	295.1 abcd	414.5 a	181.3 bcde	763.5 b
Facture	289.6 bcd	294.1 bc	137.6 ef	710.0 bc
Serenade Max	186.3 e	273.5 c	167.5 def	710.4 bc
DSD	283.5 cd	382.9 ab	183.4 abcde	812.3 ab
TST	386.9 a	282.2 c	248.2 ab	562.2 c
FSF	341.8 ac	409.6 a	206.8 abcd	682.4 bc
SSS	378.6 a	284.9 c	171.6 cdef	733.1 b

LSP	287.9 bcd	283.9 c	237.8 abc	953.0 a
ANOVA[z]	**p=0.0003**	**p=0.0003**	**p=0.0001**	**p=0.0002**