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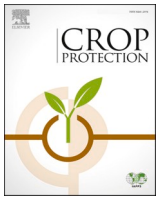

Efficacy, persistence and residue levels of fungicides for Botrytis control in wild blueberry

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ABSTRACT

Botrytis blossom blight disease is one of the major challenges to wild blueberry production with annual losses frequently exceeding 20%. In this study, the effect of different fungicide treatments on Botrytis blight development and yield, as well as the mobility and persistence of these fungicides within flower tissues, and fruit of wild blueberries were evaluated under field conditions. This multi-year trial examined five different fungicides (Switch®, Luna Tranquility®, Merivon® Xenium, Propulse®, and Miravis® Prime) each one applied twice at 7-10-day interval. Fungicide quantification in the floral and berry tissues was conducted using a modification of the QuEChErs extraction method and analyzed with GC-MS and HPLC-MS. All the treatments except Switch® reduced disease incidence by over 78 % and severity by over 40 %, compared to the control plots. Switch® and Miravis® Prime reduced both incidence and severity by over 64 % compared to the control plots. Luna Tranquility®, Merivon® Xenium, and Propulse® reduced incidence by at least 47 % and severity by 51 % compared to the control plots. Berry yields were higher in Switch®, Luna Tranquility® and Miravis® Prime treated plots with at least a 19% increase in yield compared to the control plots. The mean concentration of all quantified fungicides was higher in the corolla compared to the gynoecium and the androecium sample areas. Fungicides were persistent and concentrations were sufficient to suppress *Botrytis cinerea* at fruit set (10 days post application) with no residue detected in harvested berries, except prothioconazole-desthio.

1. Introduction

Wild blueberries are an economically important crop in Canada with annual production often exceeding 160 million kg. The crop is native, and its production is limited to Northeastern part of North America, specifically, the maritime region of Canada and the state of Maine, US (Yarborough, 2012; Hanes and Waring, 2018). The production system is unusual with the reliance on naturally occurring populations of diverse *Vaccinium* spp. genotypes, with no planting of selected cultivars, and no tillage practices.

The production of wild blueberries is faced with many disease challenges including Monilinia and Botrytis blights (Delbridge et al., 2011; Percival, 2013; Abbey et al., 2021). Botrytis blight caused by *Botrytis cinerea* is a major disease of wild blueberries which can result in significant annual yield loss with infection levels as high as 40% in some areas (Abbey et al., 2021). The control of this pathogen is highly dependent on fungicide application. An important product used for the management of the disease is a combination of fludioxonil and cyprodinil marketed as Switch®. Other fungicides known for Botrytis disease management include Luna Tranquility® (fluopyram and pyrimethanil) and Pristine® (pyraclostrobin and boscalid). The protection of blueberry flowers poses a persistent challenge despite the utilization of fungicides. There is a considerable risk of *B. cinerea* developing resistance to fungicides, as indicated by FRAC (2019). Reports of reduced fungicide efficacy in various crops, such as grapes and strawberries, further highlight this concern (Hahn, 2014; Grabke and Stammler, 2015; Latorre and Torres, 2012; Harper et al., 2022; Bolognesi et al., 2023). Instances of resistant *B. cinerea* isolates, particularly in wild blueberry and highbush blueberry fields, have been reported for some of the commonly used fungicides (Abbey et al., 2017; Naegele et al., 2022). The proliferation of susceptible floral tissues across fields, attributed to increased flower densities (>370 million flowers per hectare) resulting

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from enhanced nutrients and weed management, adds another layer of complexity (Percival 2013). The complex combination of flower clusters, pendulous flower orientation, and limited fungicide mobility presents considerable difficulties in disease management for wild blueberry. Moreover, the distinctive features of cyme inflorescence, inferior ovary, and bell-shaped flower structure hinder direct fungicide contact with the androecium and the pistil. This multifaceted interplay of factors elevates the challenge of disease management strategies in wild blueberry production. Finally, fungicides applied to the corolla face the challenge of senescence post-pollination, leaving developing berries unprotected. Given the complexities of the plant architecture, the quest for a systemic fungicide with enhanced mobility within the flower emerges as a necessary consideration.

Currently, many techniques are being evaluated to improve disease management. These include the adoption of biofungicides, the potential to harness molecular tools and the introduction of new active ingredients for wild blueberry disease management (Abbey et al., 2021, 2023). Adepidyn™ (pydiflumetofen, FRAC 7) is the new carboxamide and the first member of a new chemical group among the succinate dehydrogenase inhibitor (SDHI) fungicides, the phenyl-ethyl pyrazole carboxamides (FRAC, 2022). This compound has been demonstrated to possess remarkable efficacy against difficult pathogens such as *Botrytis cinerea* and *Sclerotia sclerotiorum*, (Sierotzki et al., 2017). Furthermore, fluxapyroxad, another SDHI, has recently been integrated into the wild blueberry production system. In the advancement of disease management for wild blueberries, pydiflumetofen is co-formulated with fludioxonil, while fluxapyroxad is co-formulated with pyraclostrobin, in commercial products. Despite their recent integration, there is a lack of reports on the efficacy, behavior (including mobility and persistence), and redistribution of these compounds, including pydiflumetofen, to adequately protect wild blueberry flowers. Most registered fungicides for Botrytis control in wild blueberries exhibit limited mobility within plant tissues due to their locally systemic nature (Beckerman, 2018; Lamichhane et al., 2020). Understanding the redistribution, persistence, and efficacy of these fungicides, particularly those newly registered for wild blueberry use, such as pydiflumetofen, within wild blueberry flower tissues, inspired this current study. This study was therefore conducted to evaluate the efficacy of selected fungicides for Botrytis control and assess their mobility and persistence within wild blueberry flowers and fruit.

2. Materials and methods

2.1. Site selection and experimental design

Four research trials were conducted on commercial wild blueberry fields in two years (Fox Point and Murray Siding, NS in 2018 and Debert and Mount Thom, NS in 2019). 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. A randomized complete block design with six replications was used. The plot size was 4 × 6 m with 2 m buffers between plots. Six treatments and two applications of each treatment were made (Table 1).

2.2. Fungicides and applications

The first fungicide applications were made at 10% bloom prior to visual symptoms of Botrytis and the second fungicide applications were made 7–10 days after the first application corresponds with 50% bloom. 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 8002VS nozzles at a pressure of 32 PSI (220 kPa). The volume application rate used was 250 L/ha. The recommended doses of each fungicide used are listed in Table 1.

In 2018, fungicides were applied on 7th June, and 15th June, respectively at Fox point and 28th May and 8th June, respectively at Murray Siding. In 2019, first and second fungicide application were made on 8th June, and 25th June at Debert and Mt Thom.

2.3. Sample collection, disease assessment and berry yield

For disease assessment, fifteen stems were randomly selected seven days after the initial fungicide application and 14 days after the second fungicide application had occurred. The stems were collected at 20 cm intervals along a 4 m line transect in each plot, placed in plastic bags and brought to the lab for assessment of Botrytis disease development (incidence and severity). Disease incidence was determined as the number 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 (Smith, 1998; Abbey et al., 2021). The data were expressed as a percentage of the affected flower area (disease severity).

Berries were harvested in August with a forty-time commercial wild blueberry hand rake from four randomly selected 1 m^2 quadrant in each plot. Harvested berries from each plot were weighed with an Avery Mettler PE 6000 digital balance, and the data were recorded. A 500 ml composite sample for each treatment was bagged and brought to the lab for fungicide residue analysis.

2.4. Analysis of fungicide residue using the 'quick, easy, cheap, effective, rugged, and safe' (QuEChERS) method and detection by GC-MS

2.4.1. Chemicals and standards preparation

Technical grades (>96% purity) of cyprodinil, fludioxonil and pydiflumetofen were obtained from Syngenta, Canada, fluxapyroxad and pyraclostrobin from BASF Canada, fluopyram, pyrimethanil, prothioconazole, and prothioconazole-desthio were obtained from Bayer Crop Science (Kansas City, USA), and triphenyl phosphate was obtained

Table 1
Fungicides and their application rates.

Products	Active ingredients	Product application Rates	Amount of active ingredient applied
Untreated Control			
Merivon® Xenium	Fluxapyroxad & pyraclostrobin	350 ml/ha	Fluxapyroxad 87.5 g/ha Pyraclostrobin, 87.5 g/ha
Propulse®	Fluopyram & prothioconazole	750 ml/ha	Fluopyram, 150 g/ha Prothioconazole, 150 g/ha
Miravis® Prime	Pydiflumetofen & fludioxonil	800 ml/ha	Pydiflumetofen 120 g/ha Fludioxonil, 200 g/ha
Switch ®	Cyprodinil & fludioxonil	975 g/ha	Cyprodinil, 156 g/ha Fludioxonil, 234 g/ha
Luna Tranquility®	Fluopyram & Pyrimethanil	1.2 L/ha	Fluopyram, 150 g/ha Pyrimethanil, 450 g/ha

from Acros Organics, Germany. Analytical grade acetonitrile, and toluene (Fisher Scientific, ON, Canada), and formic acid (>98%) were purchased from Sigma- Aldrich. Anhydrous magnesium sulphate ($MgSO_4$) (Sigma- Aldrich), sodium acetate (NaOAc), (Fisher Scientific), and primary-secondary amine (PSA, Cole Parmer, USA) for this analysis.

At least 5 mg of the chemical standards were dissolved in toluene to make a 1 mg/ml stock solution and stored at −18 °C. Calibration standards were prepared by an appropriate dilution of stock solution in toluene. Six different concentrations (0.005, 0.01, 0.02, 0.05, 0.5 and 1.0 μg/mL) of each compound were prepared to generate calibration curves.

2.4.2. Sample collection

Samples for fungicide mobility and persistence were obtained by collecting 50 stems at 15 cm intervals across each plot 24 h after each fungicide application and 10 days after the second fungicide application (fruit set). Stems were kept in a cooler with ice and brought to the lab. Approximately 30 fully opened flowers were removed from the top 5 cm portion of each stem, and these flowers were separated into corolla, gynoecium (ovary, style, stigma), and androecium (anther, filament) (Fig. 1).

2.4.3. Sample preparation

Sample preparation was carried out according to the method described by AOAC 2007.1 with modification (Lehotay, 2007; Walorczyk, 2014; David et al., 2016). Homogenized berry samples (ripped berry and set fruit at 10 days post fungicide application) of 15 g were weighed into a 50 ml centrifuge tube and 15 ml of 1% formic acid in acetonitrile was added. Internal standard (Triphenyl phosphate, TPP), 75 μl at 150 μg/ml was then added and the tube was vigorously shaken by hand and vortexed for 5 min. A buffer-salt mixture consisting of 6 g $MgSO_4$ and 1.5 g NaOAc was added to the tube and vigorously for 5 min. The tube was then centrifuged for 5 min at > 4300 rcf. The supernatant (4 ml) was mixed with 200 mg primary secondary amine (PSA), and 600 mg $MgSO_4$. The tube was vortexed and centrifuged for 1 min at > 4300 rcf. An aliquot (2 ml) of the supernatant was evaporated under nitrogen to at 30 °C and reconstituted in 1 ml toluene and filtered with a 0.45 μm nylon membrane for analysis on GC-MS.

For the flower samples, a scaled down method of the procedure described above was used with 100 mg of ground sample in a 2 ml Eppendorf tube. Distilled water (0.5 ml) was added to hydrate the sample for 5 min and 0.6 ml acetonitrile was added. Internal standard (TPP), 9 μl at 50 μg/ml was then added to the tube and vortexed for 2 min. A buffer-salt mixture consisting of 200 mg $MgSO_4$ and 50 mg NaOAc was added and vortexed for 2 min. The tube was then centrifuged for 2 min at > 15000 rcf. The extract (0.4 ml) was mixed with 20 mg PSA, 60 mg $MgSO_4$ and 20 mg activated carbon (for samples containing cyprodinil and pyrimethanil, activated carbon was excluded to prevent their adsorption by the carbon). The tube was shaken for 30 s, centrifuged for 1 min at >15000 rcf and an aliquot (0.35 ml) of the supernatant was evaporated under nitrogen and reconstituted in 0.5 ml of toluene for GC-MS and 0.2 ml methanol for LC-MS/MS.

2.4.4. Sample analysis

Residue analysis was performed on a Scion 456A GC–triple-quadrupole mass spectrometer (Bruker, Scion Instrument, Amundsenweg the Netherlands). Injection of 2 μl (split of 1:20) was made using a Bruker autosampler (Bruker, Scion Instrument, Amundsenweg the Netherlands). The GC separation was conducted on a 30 m × 0.25 mm × 0.5 μm capillary column. Helium (99.9% purity) at a flow rate of 1.2 mL/min was used. The oven temperature was programmed to start at 80 °C (hold for 1 min), increase to 180 °C at the rate of 25 °C/min (hold for 1 min), then increase to 310 °C at 10 °C/min (hold for 5 min). The ion source and MS transfer line temperatures of 280 °C were used. Electron ionisation energy of 70 eV was used. Selected ion monitoring (SIM) mode detecting 2–3 ions for each analyte was used.

Prothioconazole and its metabolite prothioconazole-desthio are not well suited for GC-MS instrumentation, (Kiet Ly, 2020; Pizzutti et al., 2012; Hergueta-Castillo et al., 2022), hence sample were extracted as described above and sent to The Water Quality Centre, Trent University (Peterborough, ON Canada) for analysis. The LC-MS/MS analysis was performed on an Agilent 1100 Series LC and autosampler (Mississauga, ON Canada). Samples separation was performed on a Thermo Acclaim RS LC 120 C18 column (2.2 μm, 2.1 × 50 mm) with a C18 guard cartridge, using a gradient elution and 0.1% formic acid in water and acetonitrile as mobile phases. Initial gradient was 10% acetonitrile and held for 0.5 min, increasing to 95% over 1.5 min and held for 2.5 min, returned to initial conditions after 0.5 min and re-equilibrated for 3.5 min, resulting in a total run time of 8.5 min. Injection volume was 20 μl at a flow rate of 0.55 ml min^{-1}.

Method validation was performed according to the European Union guidance criteria on analytical quality control and validation procedures for pesticide residue analysis in food and feed (SANTE/11312/2021, 2021). The linear standard curves were obtained from the

Fig. 1. A longitudinal section of the urn-shaped flower indicates the corolla (pink and white), androecium (yellow tissues) and gynoecium (green pistil surrounded by the yellow filament of androecium).

matrix-matched working standard solutions. A fortified study was carried out at levels of 0.01, 0.05, and 0.25 mg/kg to determine the recovery levels and precision of the analytical method.

2.5. Statistical analysis

Data collected on disease development and harvested berries were analyzed using the PROC GLIMMIX procedure of SAS (version 9.4, SAS institute, Inc., Cary, NC). Minitab version 19 was used for the analysis of residue concentration in samples using one-way ANOVA and repeated measures for flower samples. LSD was used for multiple means comparison at $\alpha = 0.05$. Prior to the analysis, the data set was subjected to a normality test. Residue concentrations from flower samples were transformed using square root.

3. Results

3.1. Fungicide efficacy

Following the application of fungicides, significant disease control was observed for all the treatments compared to the untreated control. In 2018 after the first fungicide application at Fox Point, Switch®, Luna Tranquility® and Miravis® Prime reduced disease incidence by more than 92 % and severity by over 90 %, compared to the untreated control. Also, Propulse® suppressed disease incidence and severity by 68 % and 65.3 % respectively (Table 2). Merivon® Xenium application resulted in complete disease control. After the 2nd fungicide application, Switch®, Luna Tranquility®, Merivon® Xenium and Miravis® Prime reduced incidence and severity by over 78 and 40 % respectively compared to the untreated control (Table 2).

At Murray Siding Propulse® and Miravis® Prime reduced incidence by more than 80 % and 69 % respectively. Disease severity was reduced by all the treatments by more than 64 % compared to the untreated control after the first fungicide application (Table 2). After the second fungicide application, all the fungicide treatments significantly reduced disease incidence and severity by over 80% except for Switch® (Table 2).

In 2019, all the fungicide treatments reduced disease incidence and severity by more than 76 and 57 %, respectively after the first fungicide application at Debert (Table 3). After the second fungicide application, all the treatments reduced disease incidence and severity by over 64 and 67 % respectively compared to the untreated control.

At Mt Thom, all the treatments reduced disease incidence and severity by over 69 and 80 %, respectively compared to the untreated after the first fungicide application (Table 3). After the second fungicide application, Miravis® Prime and Switch® effectively reduced disease incidence by 79 and 72 %, and severity by 81. and 76 %, respectively. Luna Tranquility®, Merivon® Xenium, and Propulse® reduced disease incidence and severity by more than 47 and 51 %, respectively (Table 3).

Although there was a significant treatment effect on berry yield at Murray Siding in 2018, most of the treatments had a lower yield than the untreated control except Miravis® Prime which produced higher yield (19.3 % more yield) compared to the untreated control (Table 4). In 2019, Switch® and Luna Tranquility® applications resulted in a 36.3 and 32 % yield increase, respectively compared to the untreated control at Debert (Table 4). At MT Thom, Switch®, Luna Tranquility®, Propulse®, and Miravis® Prime increased yield by 22, 25.7, 43.5 and 20.2 %, respectively (Table 4).

3.2. Residue in flower samples and berry samples

The GC-MS conditions adopted in the study was able to separate the fungicides of interest with a run time of 18.5 min (Fig. 2). Following criteria on analytical quality control and validation procedures for pesticide residue analysis in food and feed (SANTE/11312/2021, 2021), the means recoveries of the various fungicides for both berry and flower samples ranged from 59.9 to 121.6 % for the lowest spiking, 97.4–118.8% for 0.05 mg/kg and 90.4–108% for the highest concentration (0.25 mg/kg). The relative standard deviations (RSDs) were between 0.5% and 15% for all three-spiking levels indicating good repeatability (Supplementary material, Table B2 and B4). Standard curves generated using linear regression produced R^2 value > 0.98 for each fungicide standard (Table 5). The recovery and precision outcomes indicate that the method satisfied the requirements for analysis according to the European Union guidance criteria on analytical quality control and validation (SANTE/11312/2021, 2021).

The developed procedure was therefore used to evaluate the presence of the active ingredients/residue in the applied disease control product for the samples collected from the 2019 trial at Mount Thom, NS.

The mean concentrations of most the fungicides in the flower tissues were higher in samples collected 24 h after the second fungicide application (Fig. 4) than the first application (Fig. 3), except pyraclostrobin, fluxapyroxad and prothioconazole-desthio. Among the floral parts, the concentrations of all the fungicides were significantly higher in the corolla except fludioxonil in Miravis® Prime, pydiflumetofen and pyraclostrobin (Fig. 5). Interestingly, there was no significant difference in the residue concentrations among the three flower parts for fludioxonil contained in Miravis® Prime (Tables 4 and 5).

In the fruit set samples collected 10 days after the second fungicide application, appreciable amount of all the fungicides were detected, however, the concentrations of these fungicides were below their respective MRL except pydiflumetofen (Table 6). For the ripe berries harvested 59 days after the second fungicide application, none of the fungicides were detected (below the detection limited) in the berry harvested except prothioconazole-desthio (Table 6).

4. Discussion

Outcomes from this study have demonstrated that two applications

Table 2
Botrytis blight incidence (inc %) and severity (sev %) observed in wild blueberries at Fox Point and Murray Siding, Nova Scotia, after fungicide applications in 2018.

Treatment	Fox Point				Murray Siding			
	First application		Second application		First application		Second application	
	Inci (%)	Sev (%)	Inci (%)	Sev (%)	Inci (%)	Sev (%)	Inci (%)	Sev (%)
Untreated Control	12.7 a	6.66 a	4.22 b	1.86 ab	11.9 a	7.44 a	3.35 a	2.16 a
Switch®	0.15 c	0.15 c	0 c	0 c	6.21 ab	2.61 b	3.18 a	1.33 ab
Luna Tranquility®	0.69 c	0.44 c	1.58 c	1.11 bc	6.10 ab	1.98 b	0 b	0 b
Merivon® Xenium	0 c	0 c	0 c	0.00 c	7.99 ab	2.60 b	0 b	0 b
Propulse®	4.07 b	2.31 b	6.80 a	3.51 a	2.37 b	1.22 b	0 b	0 b
Miravis® Prime	0.95 bc	0.61 bc	0.13 c	0 c	3.67 b	0.59 b	0.66 b	0.16 b
ANOVA[a]	**p < 0.0001**	**p < 0.0001**	**p < 0.0001**	**p < 0.0001**	**p = 0.05**	**p < 0.0001**	**p =0.002**	**p =0.003**

[a] 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 $\alpha = 0.05$.

Table 3
Botrytis blight incidence (inc %) and severity (sev %) observed in wild blueberries at Mount Thom and Debert, Nova Scotia, after fungicide applications in 2019.

Treatment	Mount Thom				Debert			
	First application		Second application		First application		Second application	
	Inci (%)	Sev (%)	Inci (%)	Sev (%)	Inci (%)	Sev (%)	Inci (%)	Sev (%)
Untreated Control	45.4 a	43.4 a	11.5 a	10.6 a	20.2 a	9.90 a	3.57 a	1.31 a
Switch®	12.9 cd	10.3 c	0.92 b	0.46 b	4.42 b	1.32 b	0.60 b	0.05 b
Luna Tranquility®	21.0 bc	19.3 b	0.37 b	0.06 b	6.26 b	3.20 b	0.32 b	0.17 b
Merivon® Xenium	23.9 b	20.9 b	0.53 b	0.09 b	7.12 b	2.05 b	0.56 b	0.56 ab
Propulse®	16.9 bcd	15.1 bc	3.56 b	2.04 b	3.20 b	0.91 b	0.83 b	0.11 b
Miravis® Prime	9.66 d	8.09 c	0.96 b	0.24 b	3.87 b	1.14 b	0.64 b	0.04 b
ANOVA[a]	**p < 0.0001**	**p < 0.0001**	**p < 0.0001**	**p < 0.0001**	**p < 0.0001**	**p < 0.0001**	**p=0.041**	**p=0.05**

[a] 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 $\alpha = 0.05$.

Table 4
Harvestable berry yield ($g.m^{-2}$) observed from wild blueberry field after fungicide applications.

Treatment	2018		2019	
	Fox Point	Murray Siding	Mount Thom	Debert
Untreated Control	499.08	284.91 ab	238.09 bc	529.75 b
Switch®	360.09	254.26 bc	291.66 ab	722.00 a
Luna Tranquility®	327.23	214.16 cd	299.31 ab	699.35 a
Merivon® Xenium	327.37	212.32 cd	213.59 c	576.43 b
Propulse®	335.68	191.63 d	341.63 a	537.41 b
Miravis® Prime	369.21	339.86 a	286.27 ab	508.27 b
ANOVA[a]	**NS**	$p < 0.0001$	**p**=0.0046	**p < 0.0001**

[a] 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 $\alpha = 0.05$.

of fungicides can be an effective means of managing Botrytis blight in wild blueberries. In this study, the variation in disease development between the two years and among the various trials can be attributed to the difference in environmental conditions. In 2019, there were many infection periods that favored Botrytis blight development during the flowering period compared to 2018, consequently, higher levels of disease development were observed in 2019. Another factor that contributed to the low disease levels observed in 2018 there was the occurrence of a severe frost which destroyed many floral tissues during bloom (Supplementary material, Tables A3-A4, Figures A1).

All fungicides used applications in this study, including the newly registered active ingredients consistently resulted in low Botrytis blight development compared to the untreated control in both years. This suggests that Merivon® Xenium and Miravis® Prime, which are new to the wild blueberry industry can be effective products to be added to the list of Botrytis control products used in wild blueberry production. Miravis® Prime is a relatively new product, its effectiveness in this study is consistent with studies by Abramians and Gubler (2017) and Blundell et al. (2019) who achieved effective Botrytis disease reduction in grapes they applied Miravis® Prime. Furthermore, Merivon® Xenium

Table 5
GC-MS data.

	Retention time (min)	Target Ions (m/z)	R^2
Prothioconazole	3.8	n/a	0.99
Cyprodinil	11.3	225.2	0.99
Prothioconazole-desthio[a]	3.6	n/a	0.99
Fludioxonil	12.3	248.1	0.99
Fluopyram	11.5	396.7	0.99
Fluxapyroxad	14.8	381.2	0.99
Pydiflumetofen	16.9	426.7	0.99
Pyraclostrobin	18.5	387.8	0.99
Pyrimethanil	9.01	199.2	0.99

[a] Analysis was done using LC-MS.

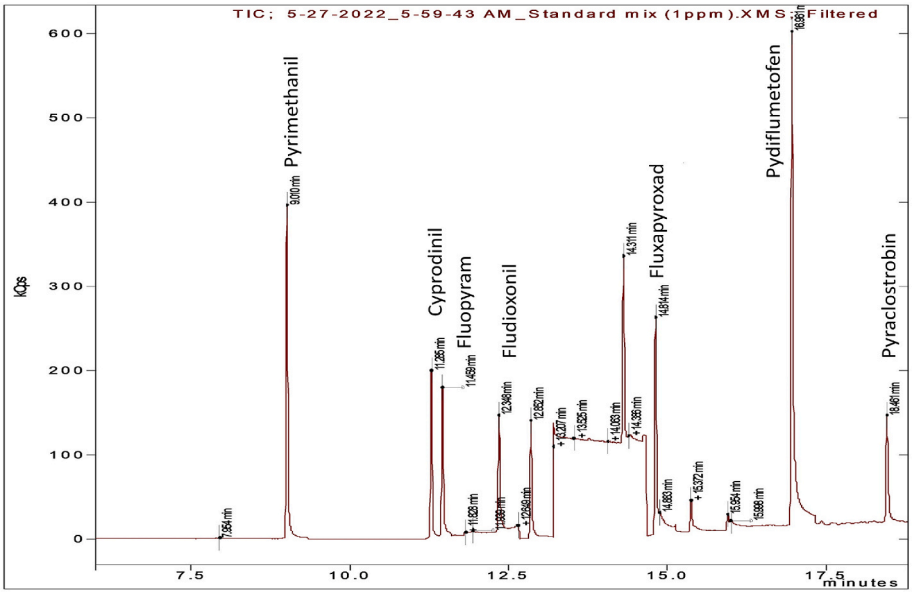

Fig. 2. Representative chromatograms (TIC mode using the quantification/target ions (Table B1) obtained from a matrix matched standard mixture of fungicides.

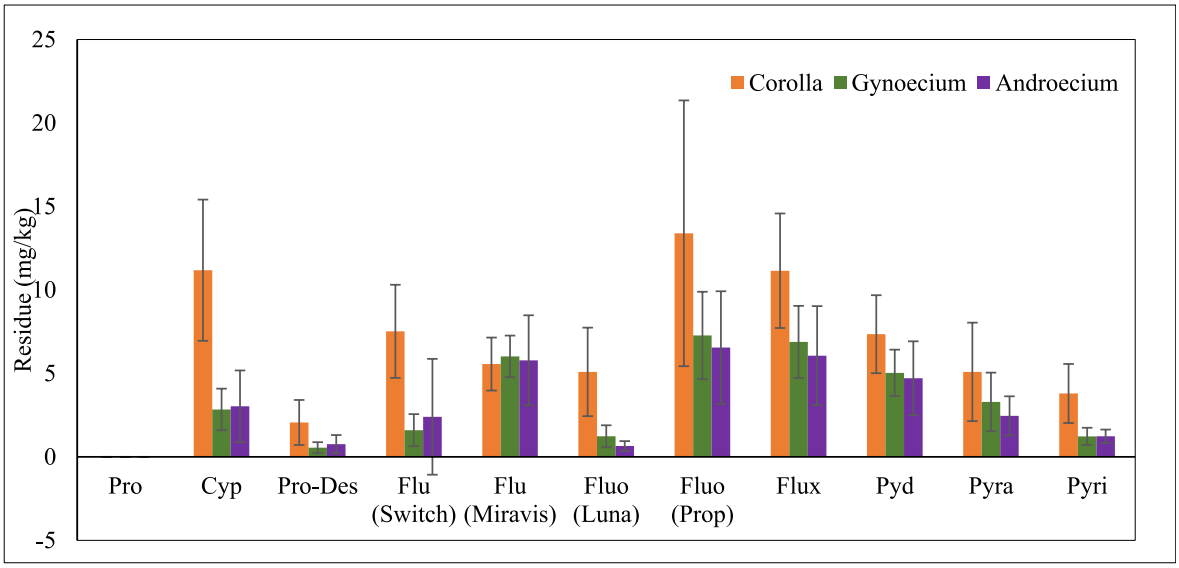

Fig. 3. Fungicide residues detected in different flower parts of wild blueberry plants 24 h after the first fungicide treatment. Concentration values are means of six replications (error bars represent standard deviation). Pro = Prothioconazole, Cyp = Cyprodinil, Pro-Des = Prothioconazole-Desthio, Flu = Fludioxonil, Fluo = Fluopyram, Flux = Fluxapyroxad, Pyd = Pydiflumetofen, Pyra = Pyraclostrobin, Pyri = Pyrimethanil.

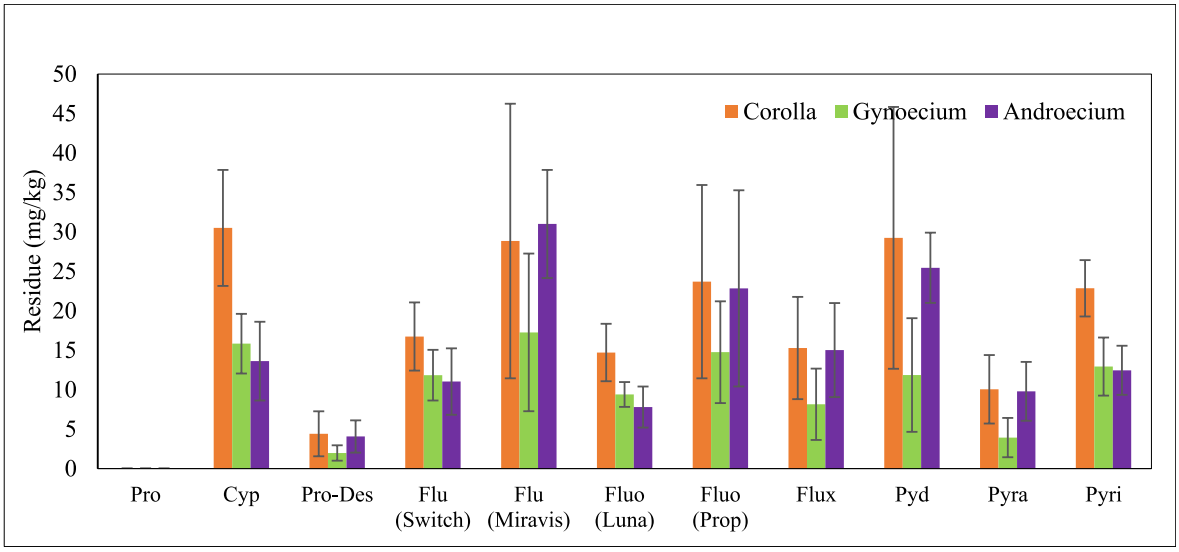

Fig. 4. Fungicide residues detected in different flower parts of wild blueberry plants 24 h after the second fungicide treatment. Concentration values are means of six replications (error bars represent standard deviation). Pro = Prothioconazole, Cyp = Cyprodinil, Pro-Des = Prothioconazole-Desthio, Flu = Fludioxonil, Fluo = Fluopyram, Flux = Fluxapyroxad, Pyd = Pydiflumetofen, Pyra = Pyraclostrobin, Pyri = Pyrimethanil.

application in strawberries was reported to effectively control *B. cinerea* (Cordova et al., 2017). These active ingredients have demonstrated high efficacy both individually or in co-formulation with other active ingredients (Uppala and Zhou, 2018). For instance, Nepal et al. (2017) reported that Merivon® was effective against Anthracnose on pomegranate. Pydiflumetofen (Miravis®), was also reported to be effective against *Fusarium* head blight and foliar diseases such as *Septoria* sp. and powdery mildew in wheat (Glynn et al., 2018). Given these, it is not surprising that these products were able to suppress Botrytis blight in this study. Although Switch®, Luna Tranquility® and Propulse® have been in use for over a decade, their effectiveness against disease development is reassuring because efficacy loss has been observed in some commercial fields.

Given that fungicide resistance development is prevalent among *B. cinerea* populations, the co-formulation of active ingredients in these products is important. Thus, Miravis® Prime contains pydiflumetofen, an SDHI and fludioxonil, a sterol biosynthesis inhibitor (Grabke and Stammler, 2015) whereas Merivon® Xenium, contains fluxapyroxad, an SDHI and pyraclostrobin, a quinone outside inhibitor (Bardas et al., 2010). The co-formulation offers high fungi control activity and presents an in-built resistance management strategy. Although the different fungicides possess different modes of action, all these active ingredients but fludioxonil are classified as medium to high-risk fungicides (FRAC, 2022). Therefore, it will be prudent that these products are applied in rotation/mixed with fungicides from other FRAC groups to minimize the development of resistance development in the pathogen population.

Berry yield from this study varied among the various trials. However, it is important to note that the application of Switch®, Luna Tranquility® and Miravis® Prime increased berry yields by at least 19% compared to the untreated control. Although berry yield increase was not statistically significant in some trials, disease development together with, other parameters such as berry yield are important in determining

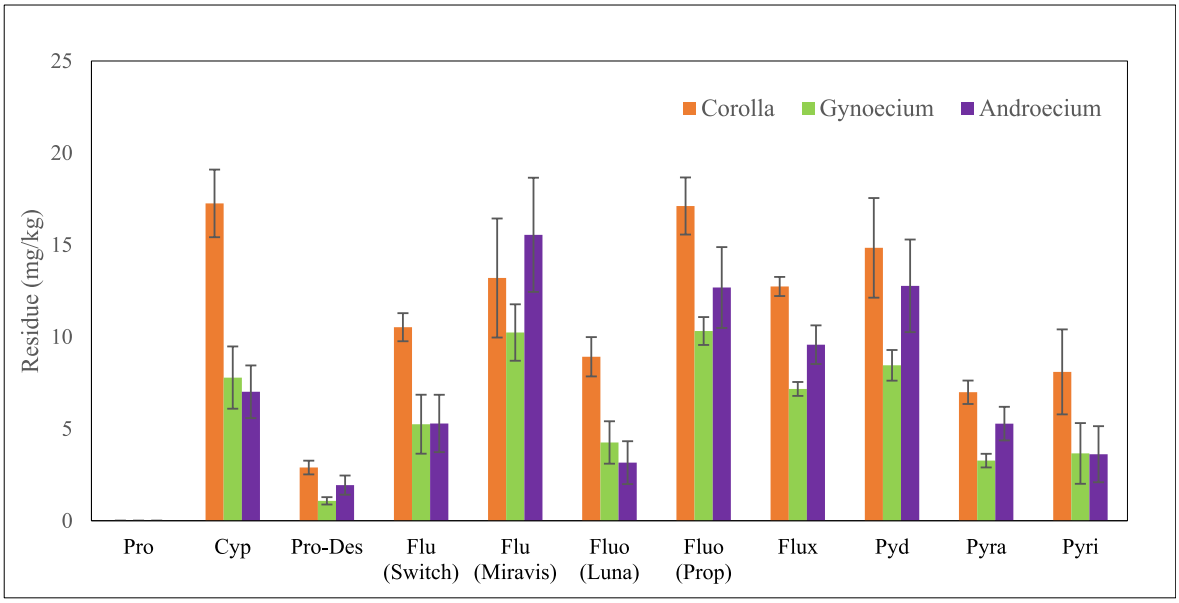

Fig. 5. Average of the two flower sampling times analyzed as repeated measures. Concentration values are means of six replications (error bars represent standard deviation). Pro = Prothioconazole, Cyp = Cyprodinil, Pro-Des = Prothioconazole-Desthio, Flu = Fludioxonil, Fluo = Fluopyram, Flux = Fluxapyroxad, Pyd = Pydiflumetofen, Pyra = Pyraclostrobin, Pyri = Pyrimethanil.

Table 6
Fungicide residue detected in fruit set (10 days post second fungicide application) and harvested berries from wild blueberry plants treated with commercial fungicides. Concentration values are means of six replications (standard deviation).

Fungicides	MRL (mg/kg)	Fruit set (mg/kg)	Berry
Cyprodinil	8.0	0.764 (0.15)	ND
Fludioxonil (Switch®)	4.0	0.314 (0.18)	ND
Fludioxonil (Miravis ® Prime)	4.0	0.034 (0.01)	ND
Fluopyram (Luna Trianquilty®)	7.0	0.122 (0.04)	ND
Fluopyram (Propulse®)	7.0	0.145 (0.02)	ND
Fluxapyroxad	7.0	0.071 (0.02)	ND
Pydiflumetofen	0.01	0.290 (0.03)	ND
Pyraclostrobin	4.0	0.155 (0.04)	ND
Pyrimethanil	8.0	0.467 (0.06)	ND
Prothioconazole-desthio	0.01	0.005 (0.005)	0.001 (0.0003)
Prothioconazole	0.01	ND	ND

ND: Not detected, MRL: maximum residue limit.

efficacy due to plant-to-plant variation and variation in disease incidence, severity, and environmental conditions.

In recent times, a satisfactory and adaptable sample preparation technique known as QuEChERS (quick, easy, cheap, effective, rugged and safe) has been well accepted for pesticide analysis due to its ability to extract multi residues from foods and environmental samples with little modification (Banerjee et al., 2012; Wang et al., 2014; He et al., 2015). In this study, a modified QuEChERS method was adopted and validated according to the European Union guidance criteria on analytical quality control and validation procedures (SANTE/12682/2019, 2020). The parameters for the validation of method included limits of detection and quantification, recovery (trueness, accuracy), within laboratory repeatability (intraday precision), reproducibility (interday precision) and matrix effect.

The matrix-matched calibration curves were used to establish the linearity with coefficients of determination higher than 0.99. The LODs and LOQs values of the various fungicides showed that the sensitivity of the method was below the MRLs set by the EU and as such, the developed method was effective and appropriate for monitoring the fungicide residues studied in blueberry samples.

The recovery of fungicides was within the range of 70–120% for the analytes, except for pydiflumetofen in the flower sample modification. The developed method was validated in terms of precision and accuracy. Using the RSD from the recovery studies, the precision values ranged between 3% and 15% which demonstrates good methodology according to SANTE/12682/2019 guidelines and literature (Maestroni et al., 2018; Constantinou et al., 2021). Although the method for suitable and acceptable for the analysis, the spiking level of 0.01 mg/kg was extremely close to the LOD for most of the fungicides, hence in the modification for the flower samples, the 0.01 mg/kg spiking were below the detection limit in some cases. At low spike levels, the matrix effects in the sample become more pronounced so very low recoveries can be obtained. Also at low spiking levels, the signal-to-noise ratio may decrease resulting in the method being susceptible to variation, hence low recovery.

In this study, significant matrix effect (ME%) was observed on all the fungicides. ME could be in the form of ion enhancement (positive value) or suppression (negative value). The ME observed in this study was largely positive with only cyprodinil and pyraclostrobin having a negative value. Interestingly, the matrix had a positive impact on pyraclostrobin in the modified flower method. The ME challenge in pesticide analysis can be compensated by dilution, use of standard addition, or matrix match calibration (Ly et al., 2020; Mahdavi et al., 2021). Additionally, ME can be reduced through extensive sample cleanup. This explains why there was minimal ME on all the fungicides except pyraclostrobin in the flower sample due to the addition of activated carbon as a component of the clean-up phase.

A higher concentration of all fungicides was detected in corolla compared to gynoecium and androecium. This is not surprising considering the corolla presumably intercepted the fungicide spray droplets from where they are distributed to the other part of the flower if they are systemic. Applied fungicides are mostly deposited on the flower and mostly on the corolla which completely houses the androecium and the pistil. Interestingly, all the fungicides, regardless of their physicochemical properties showed a similar residue distribution pattern among the three flower parts from both sampling times, except the contact fungicide fludioxonil in Miravis® Prime. The detection of these

fungicides in gynoecium and androecium could be an indication of potential mobility within the flower, however, the high concentration of these fungicides in the corolla (most Botrytis infections is visually observed) (Abbey et al., 2018), compared to the gynoecium and androecium is suggestive of limited mobility. Given the structure of the flower (androecium not exposed to direct fungicide contact), the detection of fungicides in this component of the flower could be due to the activities of the pollinating insects which may have also been a vector for these fungicides. Additionally, the volatilization of fungicides such as pyrimethanil may have contributed to the presence of these fungicides in the androecium (Houbraken et al., 2016). The similarity of the concentration between the gynoecium and androecium suggests there might not be a preferred location for the fungicides within the flower. Interestingly a similar concentration was observed for fludioxonil in Miravis® Prime among the three floral tissues. This could be so because fludioxonil is a contact fungicide and flower samples from each plot were put together in one sampling bag and brought to the lab before the flowers were separated into the various components. Therefore, cross-contamination of the gynoecium and the androecium from the corolla could have occurred.

In this study, Propulse® was included however, the residue analysis did not focus on prothioconazole and its metabolite prothioconazole-desthio. Prothioconazole is not used/registered for Botrytis control (Stehmann, 1995). The commercial product Propulse® contains fluopyram, a Botrytis control fungicide, hence it is usually used as a bridge between the *Monilinia* infection, and the *Botrytis* infection windows. Prothioconazole is not well suited for GC-MS instrumentation, (Pizzutti et al., 2012; Kiet Ly, 2020; Hergueta-Castillo et al., 2022). Prothioconazole non-volatile, polar and thermally unstable, hence they can easily decompose at high temperatures (APVMA, 2007). These properties of prothioconazole and its methabolites make it less likely to vaporize effectively in the GC column, resulting in inadequate separation. Given this challenge with prothioconazole, sample were analyzed on LC-MS instrumentation. Nonetheless, it is surprising that the parent compound, prothioconazole was below the detection limit for all the analysis whereas prothioconazole-desthio, was detected/quantified just 24 h after fungicide application. This could be attributed to challenges with extraction method and instrumentation because samples were extracted with the GC-MS validated method and shipped to a different institution to be analyzed with LC-MS. Given that the extracted samples were run on a different system, a valid conclusion cannot be made from the samples run on the LC, and as indicated prothioconazole was not the primary focus of this study.

Residues of all the applied fungicides were detected in set fruits during the 10-day post-application analysis, except prothioconazole-desthio. However, all the residues were below their corresponding MRL except pydiflumetofen (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database). During fruit set, the corolla on which fungicides are applied drops off and with the seaming limited mobility of these fungicides within the flower tissue, it is not surprising that very low levels of fungicides were detected just 10 days after the second fungicide application. From a control control point of view, all the fungicides residue detected were at, or above the EC_{50} concentrations for *B. cinerea* in previous studies with different resistance/susceptibility statuses. For instance, the residue of fludioxonil in the 10-day fruit set is 0.314 mg/kg, however, previous studies have reported 0.0047–0.0073 μg/ml and 0.1–0.2 μg/ml different *B. cinerea* isolates (Fernández-Ortuño et al., 2013; Abbey, 2017). Also, 0.314 mg/kg residue of pydiflumetofen observed was higher than the *B. cinerea* EC_{50} between 0.003 and 0.028 μg/ml reported (He et al., 2020). This suggests that the fungicide residues were persistent enough and still have the potential to inhibit *B. cinerea* growth up to fruit set.

No residue was observed at harvest (65 days fungicide application) for all the fungicides except prothioconazole-desthio. This was expected given the half-lives of these compounds reported in literature. Nonetheless, the pre-harvest interval for wild blueberry is approx. 65 days which is more than twice the half-lives of all the studied fungicides. For example, the half-life of pyrimethanil has been reported to be between 11 and 22 days in different crops including apples, table grapes and strawberries (Angioni et al., 2006; Szpyrka Szpyrka and Walorczyk, 2013). Also, cyprodinil was reported to have a half-life ranging between 9 and 20 days (Zhang, et al., 2015) while fluopyram and pyraclostrobin have been reported to have a half-life of less than 10 days in different crops even when double dose application is made (Fantke et al., 2014).

In conclusion, the results from this study provide strong evidence of the effectiveness of Switch®, Luna Tranquility®, Propulse® and newly introduced products, Miravis® Prime and Merivon® Xenium for Botrytis blight control in wild blueberry fields. The application Luna Tranquility® resulted in increased berry yield. Miravis® Prime and Merivon® Xenium can provide an alternative disease control option for growers.

The concentrations of fungicides in fruit set were high enough to adversely suppress *B. cinerea*. Fungicide concentrations were higher in the corolla than in the gynoecium and the androecium which is suggestive of limited mobility.

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CRediT authorship contribution statement

Joel Abbey: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **David Percival:** Conceptualization, Funding acquisition, Project administration, Supervision. **Laura Jaakola:** Supervision, Writing – review & editing. **Samuel K. Asiedu:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2024.106633.

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