EVALUATING FOLIAR FUNGICIDE EFFICACY, SENSITIVITY AND PERSISTENCE TO IMPROVE MANAGEMENT OF WILD BLUEBERRY (*Vaccinium* spp.) FOLIAR DISEASES

by

Alex W. Cornel

Submitted in partial fulfilment of the requirements

for the degree of Master of Science

at

Dalhousie University

Halifax, Nova Scotia

August 2023

Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Wild blueberry foliar pathogens *Thekopsora minima* and *Sphaerulina vaccinii*, indirectly reduce wild blueberry yields. Determining alternative fungicide options for wild blueberry growers is required due to deregistration of chlorothalonil in Canada. Field trials in 2021 discovered plants treated with prothioconazole + benzovindiflupyr had 49% less mean disease compared to the untreated, and 17% greater mean leaf number than all other treatments. *In vitro* fungicide sensitivity was assessed by calculating the effective concentration which inhibits fungal growth by 50% (EC_{50}). The prothioconazole-desthio EC_{50} for *S. vaccinii* was significantly lower than all other fungicides. Benzovindiflupyr was highly effective at inhibiting *T. minima* urediniospore germination, with EC_{50} being 98% lower than other fungicides. Fungicide residue in leaf tissues were assessed 0, 7, 14 and 30 days after application using GC-MS and LC-MS. Thirty days after application, fungicides were still detected, with benzovindiflupyur being detected at the greatest concentration, with pydiflumetofen, mefentrifluconazole and prothioconazole-desthio in decreasing concentration. The results from this study outline fungicides that can effectively manage foliar pathogens at a field scale, how fungicides inhibit fungal growth in-vitro for these two predominant pathogens and the duration of these fungicides within the leaf tissue. These results can help guide fungicide selection and application timing decisions within the wild blueberry industry.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ANOVA – Analysis of Variance
a.i. - Active ingredient
cm – Centimeter
DAA – Days after application
DMI – Demethylation inhibitor
EC_{50} - Effective concentration that inhibits mycelial growth by 50%
g – Gram
GC – Gas chromatography
GCB – Graphitized carbon black
ha - Hectare
kg - Kilogram
kPa - Kilopascal
L – Liter
LC – Liquid chromatography
LOD – Limit of detection
LOQ – Limit of quantification
m - Meter
mg - Milligram
mL - Milliliter
mm- Millimeter
MS – Mass spectrometry
N – North
NDVI – Normalized difference vegetative indices
PDA - Potato dextrose Agar
r - Coefficient of correlation
RH - Relative humidity
SDH - Succinate dehydrogenase
SDHI - Succinate dehydrogenase inhibitor
SPE – Solid phase extraction
UAV – Unmanned aerial vehicle
W - West
µg – Microgram
uL - Microliter
ºC - Degrees Celsius
® - Registered trademark
% - Percent
± - Margin of error of a quantity
ME – Matrix effect
< - Less than
> - More than

ACKNOWLEDGMENTS

I would like to thank a number of individuals for their contribution to this thesis and my time at the Dalhousie Agricultural Campus.

Firstly, I would like to thank my advisor, Dr. Percival, for challenging and supporting me throughout my time working with the Wild Blueberry Research Program. Thank you for helping me advance my knowledge and skills in the horticultural field and as a researcher.

I would also like to thank my committee members, Dr. Oudemans and Dr. Polashock for providing me the opportunity to discuss problems or concerns I was having with my research, despite being at a distance in New Jersey. As well, thanks to Dr. Wang-Pruski for your timely revisions and upbeat joy that you bring to the Department.

I would also like to thank the technical staff of the Department of Plant, Food and Environmental Sciences who willingly assist graduate students. Also, other members of the Wild Blueberry Research Program, Joel Abbey, Kenneth Anku and Joel Langdon for their assistance, guidance and humour. Your support was greatly appreciated.

Bragg Lumber Co. and WBPANS for their financial support of this research, as well as their support for other research within the wild blueberry industry. This work was partially supported by the Natural Sciences and Engineering Research Council project CRDPJ 507170-16 and through the Mitacs Accelerate program (Application Ref: IT29412).

The Water Quality Center at Trent University for their analysis of select fungicide active ingredients and support in the preparation of extracted samples.

Thank you to my parents, Rob and Heidi, for their continued support, encouragement and love. Since I was young, you instilled in me the value of working hard, helping others and having a sense of humour. These lessons are invaluable.

And lastly, to my girlfriend Laura, I thank you very much for always being there to listen and talk me through difficult situations, while also being there to share the successes. You have helped me along the way more than you know.

CHAPTER 1

INTRODUCTION

1 Introduction

The forests of eastern Canada and United States are the natural habitat of the native Ericaceae species', *Vaccinium angustifolium* Aiton and *V. myrtilloides* Michx., which make up commercial wild blueberry fields (Privé et al., 2012). The *Vaccinium* genus is highly diverse and has multiple species that produce edible fruit, such as wild blueberries, highbush blueberry, cranberry, lingonberry, bilberry and huckleberry (Vander Kloet, 1988). Wild blueberries have become an important part of the rural economy in eastern Canada, with this fruit being desired around the world for its flavour, versatility and health benefits. Today, approximately 98% of the harvested berries are individually quick frozen shortly after harvest and destined for the export market (Percival & Burnham, 2009).

One of the biotic factors that impact wild blueberry yields is the development of diseases. Wild blueberry plants are affected by a variety of diseases, with Monilinia blight and Botrytis blight impacting the floral buds and flowers, and foliar diseases such as blueberry leaf rust, Sphaerulina leaf spot and Valdensia leaf spot impacting the plant's foliage (Polashock et al., 2017). *Thekopsora minima* P. & H. Sydow, *Sphaerulina vaccinii* Ali, Hildebrand & Abbasi (previously *Septoria spp*.) and *Valdensia heterodoxa* are the causal organisms for foliar diseases, blueberry leaf rust, Sphaerulina leaf spot (previously Septoria leaf spot) and Valdensia leaf spot, respectively (Polashock et al., 2017). Other foliar diseases exist, however; in comparison, they are of minimal concern (Hildebrand, Renderos & Delbridge, 2016). Foliar diseases were once of minimal concern; however, in the past 20 years they have become increasingly problematic (Percival & Dawson, 2009).

Alone, these diseases can cause significant yield losses when unmanaged in the vegetative year of production of the production cycle (Dawson, 2009; Dawson and Percival, 2009).

Chemical control methods such as fungicides have been the predominant method for managing foliar diseases during the vegetative year. Chlorothalonil (Bravo® ZN) was once a commonly used fungicide for its effective management of foliar diseases, especially when used in combination with prothioconazole (D. Percival, personal communication, Dalhousie University, NS, CA). However, the European Union, who is a large importer of Canadian wild blueberries, raised questions over the safety of chlorothalonil regarding human health. Most recently, wild blueberries have been removed from the Bravo® ZN label and as of 2022 chlorothalonil was deregistered for use in Canada (Pest Management Regulatory Agency, 2022). In recent years, new fungicides have become registered for managing wild blueberry foliar diseases, and novel fungicides may become registered in the near future. Given the changes to fungicide options available for managing foliar diseases, a thorough evaluation of the efficacy, sensitivity and persistence of current and novel fungicides is required.

1.1. Hypothesis and Objectives

1.1.1 Hypothesis

In general, the use of fungicides for managing foliar diseases is well documented on a variety of crops. When fungicides are used to managed foliar diseases, a reduction in disease incidence, and an increase in plant health, leaf area, leaf number, floral bud, yield components, and yield have been observed. It is anticipated that foliar fungicides used for managing wild blueberry foliar diseases will reduce disease incidence, resulting in an increase of these cumulative physiological measurements.

Growers are using a combination of a demethylation inhibitor (DMI), prothioconazole (Proline®), and a succinate dehydrogenase inhibitor (SDHI), benzovindiflupyr (Aprovia®), for control of foliar diseases on wild blueberries. The combination of other DMI and SDHI fungicides have shown to reduce foliar diseases on many other crops as well. In particular, prothioconazole combined with benzovindiflupyr is commonly used on wheat for management of Septoria leaf spot, leaf rust and stripe rust. The active ingredients being tested in this project include, difenoconazole, mefentrifluconazole, pydiflumetofen, folpet, metconazole, fluoxastrobin and the novel florylpicoxamid have reduced leaf spot diseases and rust diseases on other crops. Likewise, it is anticipated that the use of DMI and SDHI fungicides together will reduce *T. minima*, *S. vaccinii,* and *V. heterodoxa* infection in the lowbush blueberry.

Fungicide resistance has been documented for a variety of fungicides, including ones that are registered on wild blueberries. Factors that influence the development of fungicide resistance include quantity of fungicide used, number of applications per growing season and species biology. Fungicides are typically only applied once, or at most twice, for managing foliar diseases. Considering this, it is anticipated that *T. minima* and *S. vaccinii* will not have reduced sensitivity to fungicides that have been used to manage foliar diseases such as prothioconazole and benzovindiflupyr.

Fungicide persistence in leaf tissues has been evaluated on other crops around the world, however; the persistence of some of the fungicides used in the present study have not been documented. The rapid conversion of prothioconazole to prothioconazole-desthio has been observed and is expected to occur in wild blueberry leaves also. Based on other

dissipation rate studies, it is expected that prothioconazole-desthio will persist longer than prothioconazole and other fungicides tested.

The specific hypotheses for this study were:

1. Foliar pathogens such as *S. vaccinii* and *T. minima*, which impact wild blueberry production in the vegetative year, can be managed using fungicides, resulting in improvements in leaf retention, NDVI, yield components and harvestable berry yield.
2. When assessing fungicide sensitivity *in vitro* using isolates of *S. vaccinii* and *T. minima* collected from commercial fields, isolates will be sensitive to active ingredients used in the field trials, and there will be a positive correlation between isolate sensitivity and resulting fungicide efficacy.
3. Fungicide active ingredients which provide high levels of disease management in the field trial section of the study, will persist longer and at higher concentrations in the plant, compared to active ingredients which poorly manage diseases.

1.1.2 Objectives

1) To evaluate the efficacy of foliar fungicides for the management of *S. vaccinii*, *T. minima* and *V. heterodoxa* using field trials.

2) Examine the *in vitro* sensitivity of *S. vaccinii* and *T. minima* to active ingredients prothioconazole, prothioconazole-desthio, benzovindiflupyr, mefentrifluconazole, and pydiflumetofen, all of which are either currently used or could be used in the future.

3) Quantitatively determine the persistence and dissipation rate of prothioconazole, prothioconazole-desthio, benzovindiflupyr, mefentrifluconazole, and pydiflumetofen

using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) analytical techniques.

1.2 Literature Review

1.2.1 Wild Blueberry Production

North America is home to a plethora of plant species that fall within the *Vaccinium* genus, some of which produce edible berries. Many of these species belong to the subgenus *Cyanococcus* which is where *V. angustifolium* and *V. myrtilloides* are phylogenetically placed. *V. angustifolium* and *V. myrtilloides* are native species that comprise commercial wild blueberry (occasionally called lowbush blueberry) fields, with *V. angustifolium* being the predominate species (Kinsman, 1993). Highbush blueberries (*Vaccinium corymbosum*) are separate from wild blueberries, due to differences in genetics, physiology, management practices, nutrition, and flavour (Retamales & Hancock, 2012). Highbush blueberries have undergone intense selective breeding to enhance yield, thermal tolerance, firmness and flavour, while wild blueberries have not (Lobos & Hancock, 2015). Over the last century, the ease of growing, breeding and harvestability, as well as their value for health benefits and taste, have made blueberries become the most popular *Vaccinium* fruit around the world (Song & Hancock, 2011; United States Department of Agriculture, 2021).

V. angustifolium and *V. myrtilloides* are low growing plants, reaching 10 to 60 cm and forming a dense canopy when competing weeds are not present (Kinsman, 1993). They are deciduous plants, with ellipsoid glossy green leaves that turn red in late autumn. Leaves have a notably waxy epicuticular layer on the adaxial surface with stomata being absent from the adaxial leaf surface. This feature can make them more tolerant of extreme weather conditions such as drought (Glass, 2000). Flowers are white to pink, urceolate shaped, 5

mm in length with the corolla being tightly clustered around the stylet and stamens which extend towards the flower's opening (Hall et al., 1979). *V. myrtilloides* can be distinguished from *V. angustifolium* by the hairs found on the leaves and stems.

Long before commercial wild blueberry fields became prominent across northeastern North America, the indigenous peoples of the region were harvesting the fruit from the forest understory (Wood, 2008). Since then, land management practices such as removing competing vegetation, bi-annual pruning and the use of modern agricultural inputs have made wild blueberries a major agricultural commodity for this region (Kinsman, 1993). Today, the 69,000 hectare commercial wild blueberry industry in Canada exists in the provinces of Quebec, New Brunswick, Nova Scotia and Prince Edward Island, with Quebec having the largest area in production (Agriculture and Agri-Food Canada, 2022). Compared to other fruit crops, wild blueberries have the highest export value, making up about 33% of the total value of fruit exported from Canada (Agriculture and Agri-Food Canada, 2022). The United States, European Union, and Japan are the largest importers of frozen wild blueberries, desired for their versatility, flavour and health benefits (Agriculture and Agri-Food Canada, 2022).

Commercial fields are created by clear-cutting forested areas and levelling the ground, allowing the native blueberry plants to utilize available sunlight. Their underground rhizomes are shallow, fibrous systems which generate new vertical shoots when mature, allowing the original plant to spread its periphery to surrounding soil when available (Kinsman, 1993). The dispersal of seeds is how new clones form (Government of New Brunswick Agriculture, Aquaculture and Fisheries, 2010). Each connected rhizome system and its stems is considered a clone, having a different genetic make up than

neighbouring rhizome systems (Kinsman, 1993). Within each species, the genetically distinct clones play a vital role in berry production because the wild blueberry plants require cross-pollination from other clones to produce berries (Eck, 1996). Once a commercial field is established, the variety of clones and species creates dense canopy made up of mosaic of clones (Kender & Eggert, 1966).

Wild blueberry plants are well adapted to their native region of northeastern North America. The soils in this region are naturally acidic resulting from their acidic glacial till parent material (Heung et al., 2021). The typical soil conditions for wild blueberry fields are acidic (pH 3.9-5.5), low in nutrients, highly leached and high in organic matter (Farooque et al., 2012; Maqbool et al., 2016). The blueberries shallow rhizome network is well adapted to the shallow, stoney soils with exposed bedrock in some places (Heung et al., 2021). In addition, plant physiological characteristics, such as having up to 85% of the biomass in the form of underground rhizomes and roots, and also thick waxy cuticles covering the leaf surface, allow this crop to survive the harsh climatic conditions of northeastern North America (Hall et al., 1972; Glass, 2000).

Following the establishment of wild blueberry fields, they are managed on a two-year or biennial production cycle. During the first year of the production cycle, referred to as the vegetative year of production, fertilizer is applied in the spring to promote shoot number and stem length (Maqbool et al., 2016). In late June and early July, there is an emergence of new vegetative growth from the rhizomes forming a dense canopy which is susceptible to foliar diseases. Floral bud initiation and growth occurs in the fall of the vegetative year and is induced by shorter photoperiod and decreased temperatures (Pescie et al., 2011). In the second year of production, known as the crop year, flowers emerge in

late May, are pollinated, berries are set and then harvested in August (White, Boyd, & van Acker, 2012). Since flowers need to be cross pollinated to be properly fertilized, pollination is a key component of commercial production. Following harvest, the stems are mechanically pruned to promote vegetative growth the next season. Burn pruning was traditionally used to prune fields, however due to the high cost of fuel and environmental impact, it is no longer used.

1.2.2 Foliar Diseases

There are several fungal pathogens that infect wild blueberry plants, with some infecting the floral tissue and berries while others infect the vegetative tissues. *Monilinia vaccinii-corymbosi* and *Botrytis cinerea* infect the floral bud and berry tissues, with *B. cinerea* also infecting the vegetative tissue on occasion. As for the foliar diseases, growers and researchers have noted an increase in leaf rust, Sphaerulina leaf spot (previously referred to as Septoria leaf spot) and Valdensia leaf spot disease over past 20 years (Percival & Dawson, 2009). Leaf rust is the most detrimental disease, due to its polycyclic disease cycle and effect on floral bud formation, Sphaerulina leaf spot (previously referred to as Septoria leaf spot) is also prevalent in most fields while Valdensia leaf spot is generally of minimal concern (Hildebrand, Renderos & Delbridge, 2016). In addition to these diseases, red leaf, powdery mildew, witches' broom, Phomopsis canker and Exobasidium leaf spot can occasionally be found in wild blueberry fields (Polashock et al., 2017).

Blueberry leaf rust, caused by *Thekopsora minima* (synonym *Pucciniastrum vaccinii*) (Pfister, Halik, & Bergdahl, 2004), is the most detrimental foliar disease to wild blueberries in eastern Canada. It has been suggested that in North America there are two closely related species, *Naohidemyces vaccinii* in the western part of the continent and *T.*

minima in the eastern part (Polashock et al., 2017). *T. minima* is present in highbush blueberry growing regions around the world and can now be found in western USA, parts of Europe, South America and Australia (Huarhua et al., 2020; Shands et al., 2018; Simpson et al., 2017; Wichura, Brand, & Böhm, 2020). In the wild blueberry fields of eastern Canada, spring conditions signal *T. minima* teliospores found on the previous years leaf litter to germinate, producing basidia and basidiospores (Figure 1.1). Basidiospores are released carried by wind to infect eastern hemlock (*Tsuga canadensis*) needles in neighbouring woodlands. In late June, small yellow aecia can be found on the underside of the hemlock needles. Aeciospores released from aecia are windblown and infect the present year wild blueberry leaves (Polashock et al., 2017). By late July, distinct yellow uredinia pustules, often used for disease identification, form on the abaxial side of the leaves. Urediniospores continue to be released from uredinia during the months of August and September which can infect more of the present crop leaves. In autumn, black telia develop on infected leaves which act as their over-wintering structure (Figure 1.1) (Hildebrand et al., 2016).

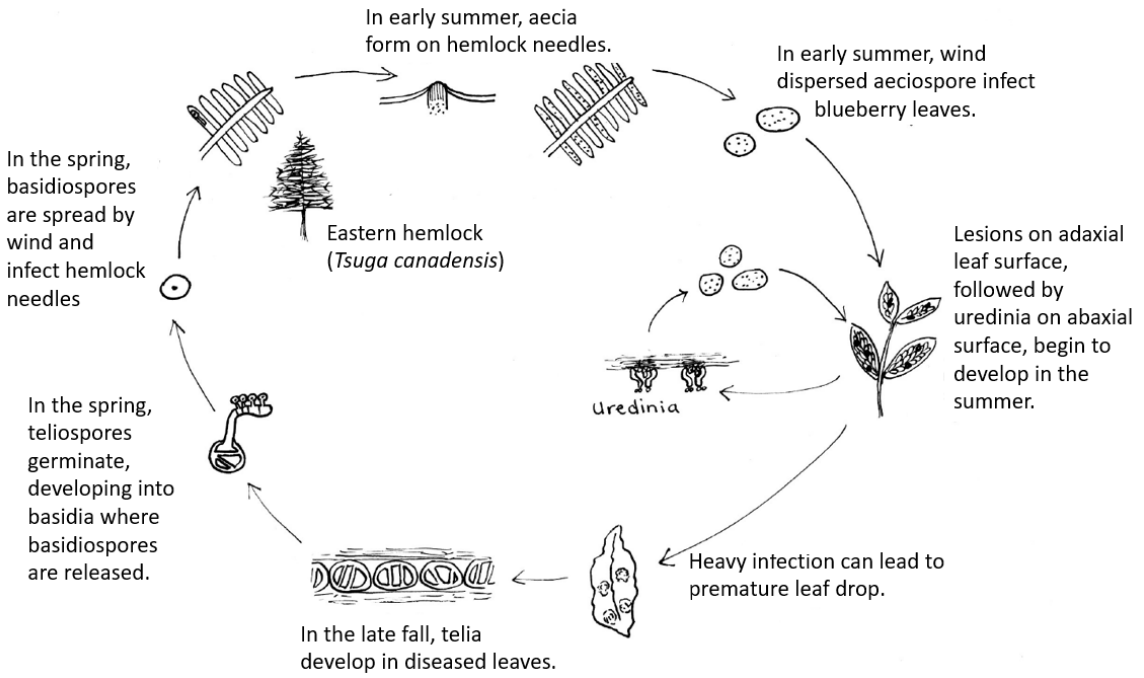

Figure 1.1 The heteroecious life cycle of *Thekopsora minima* on wild blueberries.

In recent years, research has been conducted to phylogenetically identify and classify the causal organism responsible for Septoria leaf spot disease found on wild blueberries. A species from the *Septoria* genus was thought to be the causal organism based on morphology and preliminary molecular characterization (Hildebrand et al., 2010). The phylogeny and taxonomy of the Septoria genus has recently undergone some adjustments which may influence the placement of organisms within or outside of the genus and neighbouring genera (Quadvlieg et al., 2013). In 2021, further molecular analysis of this organism found that it did not belong to the *Septoria* genus, but rather to the closely related anamorphic *Sphaerulina* genus (teleomorph: *Mycosphaerella*) and was given the name *Sphaerulina vaccinii* Ali, Hildebrand & Abbasi (Ali et al., 2021). With this is mind, additional causal organisms with similar disease symptoms could be present in the field and may not yet be formally identified and classified. Sphaerulina stem canker has also

been documented as a disease associated with *S. vaccinii*, however, has not been observed as being related to yield loss but is a key source of inoculum (Hildebrand et al., 2016).

At a field scale, *S. vaccinii* pycnidia found on the previous years fallen leaves or stem cankers begin to release conidia which infect the present years crop in early June, with peak sporulation time coinciding with the blueberry bloom period (Figure 1.2) (Ali et al., 2021). Diseased leaves become noticeable in fields in late June with lesions appearing on the abaxial leaf surface as tiny water-soaked lesions, before progressing into lesions on the adaxial leaf surface. Lesions are characterized by a sunken brown center with purple to red margins and usually a yellow necrotic ring surrounding the lesion (Hildebrand et al., 2016). The disease progresses throughout the season with lesions increasing in number and size. Heavily infected leaves are frequently found in the lower part of the crop canopy and lead to premature defoliation.

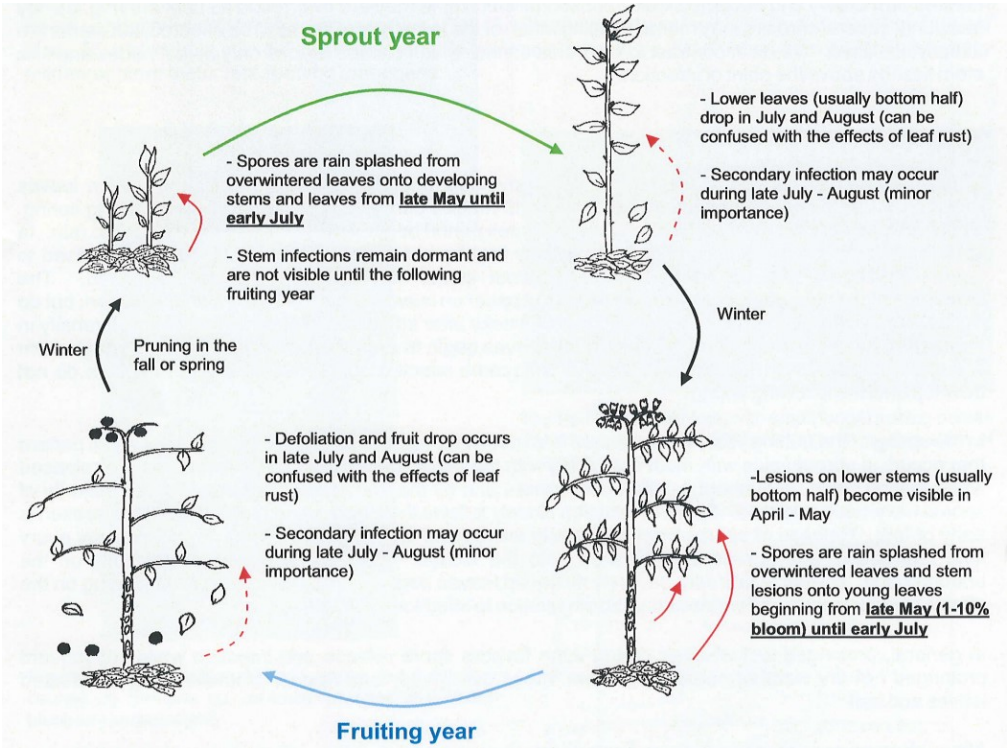

Figure 1.2 The life cycle of *Sphaerulina vaccinii* in wild blueberry (Hildebrand, Renderos, & Burgess, 2011).

1.2.3 Disease Impact on Plant Physiology

Blueberry leaf rust and Sphaerulina leaf spot pose a greater problem to wild blueberry growers in the vegetative year of production compared to the crop year (Dawson, 2009). Diseases reduce yields by limiting photosynthetic leaf area, inhibiting the amount of carbon fixed, utilizing host carbohydrates for metabolism, causing premature defoliation and reduced floral bud formation (Dawson, 2009; Garnica et al., 2013; Ojiambo, Scherm, & Brannen, 2006) (Figure 1.3A and B). Premature defoliation reduces the ability for the plants to allocate carbohydrates to floral bud tissues at the end of the growing season, reducing the next years yields (Roloff, Scherm, & van Iersel, 2004). It has been observed that leaf rust and Septoria leaf spot can reduce net carbon exchange rate by 50%, leaf respiration rate by 20% and leaf number by 50% (Dawson, 2009). Alone, these diseases

can cause yield losses of up to 100% when left unmanaged in the vegetative year of production (Dawson, 2009; Dawson and Percival, 2009).

Southern highbush blueberries are susceptible to *Septoria albopunctata* which is the causal organism for Septoria leaf spot on southern highbush blueberries, but not found on wild blueberries, is known to negatively impact the crops growth and reproduction. Roloff, Scherm & van Iersel (2004) found that *S. albopunctata* reduced the net assimilation rate of highbush leaves by half when disease severity was 20%, and to a rate of approximately zero when severity was greater than 50%. Premature leaf drop, as a result of high disease incidence, was also found. As for the relationship between disease severity and floral bud number, Ojiambo et al. (2006) found that disease severity had a significantly negative relationship with floral bud number per shoot. Negative effects of Septoria leaf spot infection were also seen on the total fruit weight per shoot. The findings of this study are useful for extending the concepts to wild blueberries and comparing to what Dawson (2009) observed.

Rust pathogens are known to utilize carbohydrates in the leaf tissue for their own metabolic processes (Kemen, Agler, & Kemen, 2015). It is also understood that rust pathogens can interrupt the carbon source/sink dynamics within a plant (Simón et al., 2020). Most rust causing species have an organ specially suited for intercellular movement within plant tissues called the haustorium (Voegele & Mendgen, 2003). The haustorium is a mycelial structure that branches out within the leaf tissue to increase the surface area between the fungus and plant cells. Surrounding the haustoria cells is the extrahaustorial matrix mixture, comprised of carbohydrates and proteins, which functions as a buffer between invading fungal cells and host cells, allowing the fungi to cause minimal damage

to the host (Voegele & Mendgen, 2003). Once establishing within the host, rust pathogens can utilize their invertase enzymes, which catalyses the breakdown of sucrose, having the ability to make the infection site appear to the plant as a carbon sink (Voegele et al., 2006). In doing so, the plant will allocate carbohydrates to the infection site, benefiting the pathogen (Voegele et al., 2006). This is thought to create a competition of resources for the plant which will decrease the quantity of resources sent to the actual plant sink organs (i.e. floral buds) (Voegele et al., 2006). Since *S. vaccinii* and *T. minima* both reduce the photosynthetic capacity of the plant or remove carbohydrates, growers need effective control options to prevent yield losses.

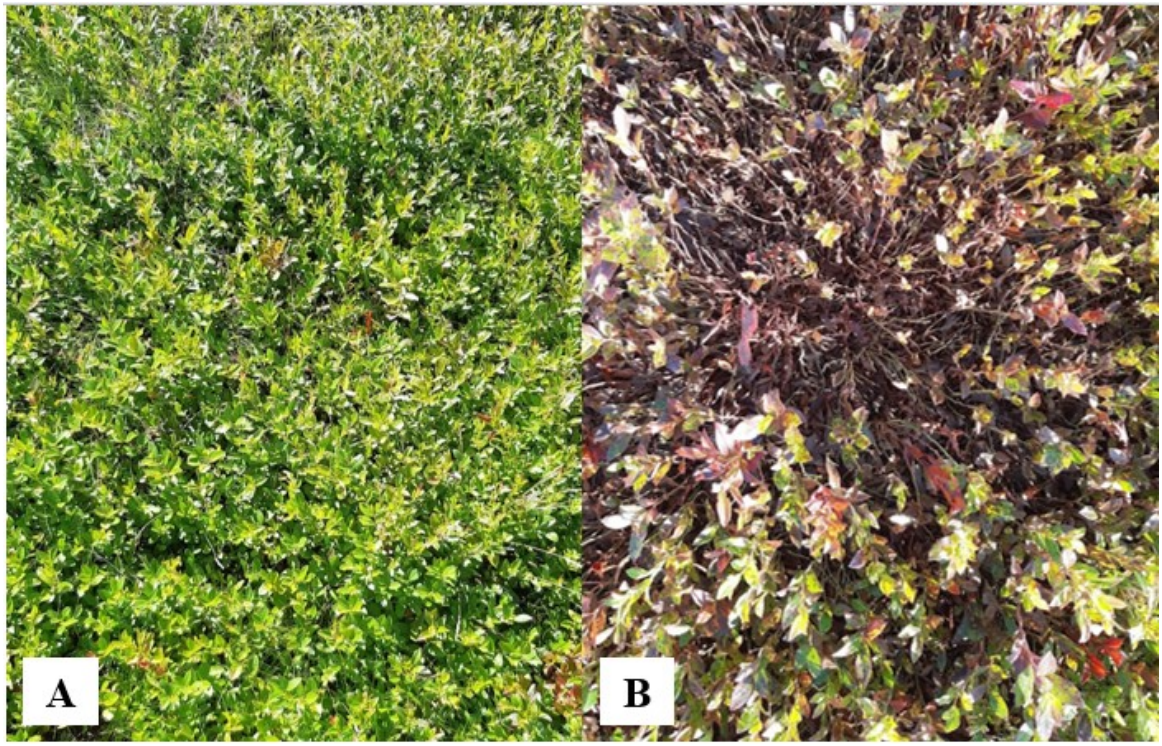

Figure 1.3 (A.) Healthy blueberry canopy on August 2, 2020 in Nova Scotia, CA, before foliar diseases became a significant concern, and (B.) diseased and defoliated blueberry canopy on September 24, 2020 as a result of primarily blueberry leaf rust, but also Sphaerulina leaf spot.

1.2.4 Chemical Management

In the vegetative year, fungicides to manage foliar pathogens are applied once during the growing season, sometimes twice if disease pressure is high. The first application is made in July to suppress *S. vaccinii* infection in the lower parts of the canopy, and also provide protection for when *T. minima* begins to appear (Hildebrand et al., 2016). This can also correspond with tip-dieback stage, which occurs naturally when vertical vegetative growth from the shoot apical meristem stops (Smagula & DeGomez, 1987). It is also at this point in time that floral bud formation is initiated and continues until the plants are dormant through the winter months. If high amounts of leaf rust are observed in vegetative year fields and weather conditions are conducive for infection, a second application is occasionally made roughly 14 days after the first application. Dawson and Percival (2009) conducted a study on wild blueberries comparing various fungicides and fungicide application times for control of leaf spot diseases and leaf rust. The study displays the requirement for effective fungicides to prevent yield losses. The researchers applied in late August of the vegetative year. They found that net photosynthesis was up to 55% greater for fungicide treatment plants compared to the untreated control. Floral bud numbers were up to 64% greater in fungicide treated plants compared to the untreated. The researchers also found that fungicide treatments increased yield by 60% over the untreated control. Chlorothalonil provided the greatest control of leaf spot and leaf rust, with boscalid and pyraclostrobin also showing effective control.

Chlorothalonil (Bravo® ZN) was once a commonly used fungicide on wild blueberries for its effective management of foliar diseases (Percival & Dawson, 2009). However, wild blueberries were removed from the Bravo® ZN label and shortly after it was

deregistered for use in Canada in 2022 (Pest Management Regulatory Agency, 2022). The fungicide received deregistration primarily due to human health concerns surrounding cancer risks to the general population from contaminated food and drinking water (Pest Management Regulatory Agency, 2022).

In the last few years, growers have switched to using a tank mix of prothioconazole and benzovindiflupyr as an alternative to chlorothalonil. This fungicide combination is commonly used around the world in wheat growing regions for management of Septoria leaf spot, leaf rust and stripe rust. Prothioconazole (Proline® 480 SC; Bayer Crop Science) is classified as a DMI which inhibits C-14 demethylase during the synthesis of ergosterols in the cell membrane (Parker et al., 2011). Benzovindiflupyr (Aprovia®; Syngenta Crop Protection Inc) is classified as an SDHI which is absorbed by growing fungal hyphae. It acts to inhibit fungal growth by blocking the activation site of succinate dehydrogenase, effectively stopping the electron transport chain process within the mitochondria of the fungal cell (Kuznetsov et al., 2018). In addition to prothioconazole and benzovindiflupyr there are other fungicides that have recently become available to blueberry growers for management of foliar diseases. Cevya® (Mefentrifluconazole; BASF) is a DMI fungicide currently registered in Canada on other fruit crops such as apples and grapes and has shown high activity against foliar pathogens *Zymoseptoria tritici* (Septoria tritici blotch on wheat) and *Puccinia striiformis* f. sp. *tritici (stripe rust on wheat)* (Carmona et al., 2020; Ishii et al., 2021). Cevya® became registered on wild blueberries in 2023 for use against Botrytis gray mold, Monilinia blight and Septoria leaf spot. Pydiflumetofen is an SDHI fungicide recently registered on wild blueberries in the product Miravis® Neo (MacDonald and Lyu, 2022). A thorough assessment of fungicide efficacy in the field, sensitivity in-vitro and

persistence in the blueberry plant is required for existing and newly available fungicides to better understand which diseases they manage and the duration for which they manage diseases.

1.2.5 Fungicide Sensitivity

With the transition of fungicides from modes of action that inhibit fungal growth through multiple pathways to those that have site-specific modes of action, there is increased risk of developing resistance (Brent & Hollomon, 2007). Factors that can influence fungicide resistance include the quantity of fungicide used, number of applications per growing season and species biology (Brent & Hollomon, 2007). Prothioconazole and benzovindiflupyr are typically only applied once per growing season for managing foliar diseases on wild blueberries, sometimes twice if conditions are conducive for infection. While these are not optimal conditions for developing resistance, prothioconazole is applied at other times in the growing season for managing *Monilinia vaccinii-corymbosi* which could place a selection pressure on populations of *S. vaccinii* and *T. minima* during other parts of their growth and reproductive cycles (Percival et al., 2017).

Fungicide sensitivity testing can be an effective method to determine the sensitivity of the organism to a particular fungicide and how that sensitivity changes over time (Russell, 2002). Fungicide sensitivity is determined by growing the fungal species on media or in the presence of fungicides at various concentrations, sometimes called a bioassay. This can be done using a variety of techniques, including spore germination tests, mycelium colony growth diameter or the optical density in a microtiter plate (Russell, 2002; Buck & Williams-Woodward, 2003; Rampersad, 2011; Sang, Popko, & Jung, 2019). The sensitivity of a pathogen to a select fungicide is typically assessed by determining the EC_{50}, which is

the concentration of fungicide that inhibits growth by 50 percent (Koller & Scheinpflug, 1987). The EC_{50} value can help indicate how effective that fungicide is at managing a particular disease in the field. It can also be used to assess the development of a fungicide resistant population (Kiiker et al., 2021). Baseline EC_{50} values can be determined prior to the widespread use of a fungicide for managing a particular disease, and then it can be reassessed at different temporal intervals to determine if the EC_{50} values have changed since it began being used in the field (Birr et al., 2021; Hsiang et al., 1997). In addition to evaluating fungicide sensitivity for fungicides registered on wild blueberries, developing an understanding of how long these systemic fungicides persist in the plant and at what concentrations, can be useful in determining their effectiveness.

1.2.6 Fungicide Persistence

Many fungicides that have become available to wild blueberry growers in recent years are systemic in nature, while many of the older fungicides were considered contacts or protectants (Augusto & Brenneman, 2012). When systemic fungicides are applied they permeate the cuticle layer and move among the leave's mesophyll cells (Satchivi, 2014). Depending on the physiochemical properties of the fungicide, localized movement into the vascular tissue and throughout the plant is possible (Satchivi, 2014). Systemic fungicides can be more effective than contact fungicides as they are less susceptible to being washed off the leaf surface and to an extent, they are able to move throughout the plant (Schilder, 2010). Within the plant, systemic fungicides dissipate into smaller metabolites, some of which can be evaluated at various time intervals after application much like the parent chemical; however, many fungicide metabolites are not yet identified (Hergueta-Castillo et al., 2023). Having the ability to determine the

concentration of fungicides within the plant tissue at various time intervals after application provides greater insight into how long they can successfully manage diseases and how often they need to be applied (Lehoczki-Krsjak et al.,2013).

Methods commonly used to assess fungicide residues in leaves, fruit or seed are more complex than bioassay methods used for determining fungicide sensitivity. Traditional methods to determine fungicide residues in soil, water or plant material was done by using ^{14}C-labeled pesticides (Dec et al., 1997). While those methods are still used today, gas chromatography coupled with mass spectrometry (GC-MS) or liquid chromatography coupled with mass spectrometry (LC-MS) are now the most widely used analytical methods and are consistently reported in the literature for assessing fungicide residues in various plant tissues (Grimalt & Dehouck, 2016; Heshmati et al., 2021; Silva, Lopez-Avila, & Pawliszyn, 2013). Many studies use these analytical techniques to determine the fungicide residues found in consumer products such as fresh fruit and vegetables to protect the consumer from any residues that may exceed safe levels (Bakirci & Hişil, 2012). Other studies have used these techniques to assess the persistence of pesticides in the leaf tissue in the field and determine degradation rates of various fungicides (Ryckaert et al., 2007). The persistence of fungicides can be influenced by physiochemical properties, fungicide formulation, environmental conditions and plant physiology, exemplifying why persistence studies need to be completed with specific fungicides on specific crops (Klittich & Ray, 2013). The results of a fungicide persistence study conducted on wild blueberries will provide the industry with an understanding of how long fungicides persist at concentrations needed to inhibit pathogen growth, which is

especially important given only one application, sometimes two, is used to manage foliar diseases.

CHAPTER 2

ASSESSING FOLIAR FUNGICIDE EFFICACY FOR IMPROVED MANAGEMENT OF WILD BLUEBERRY FOLIAR DISEASES

2 Abstract

Foliar disease of wild blueberry such as blueberry leaf rust (*Thekopsora minima*), Sphaerulina leaf spot (*Sphaerulina vaccinii*) and Valdensia leaf spot (*Valdensia heterodoxa*), can significantly reduce yield if unmanaged during the vegetative year of production. The once widely used chlorothalonil, which was used independently or in conjunction with prothioconazole, is no longer being used by many growers due to its deregistration in Canada. The objective of this study was to evaluate the efficacy of registered and new fungicides for foliar disease management by comparing leaf number, NDVI (Normalized Difference Vegetative Index), floral bud number and harvestable berry yield. Field trials were split into Protocol A and B. Protocol A and B both consisted of two field trials in commercial wild blueberry fields during the 2021 and 2022 seasons. Protocol A was used to compare fungicide combinations, whereas Protocol B was used to examine individual fungicides that have not previously been tested on wild blueberry foliar diseases. For Protocol A, the mean diseased leaves per stem for prothioconazole + benzovindiflupyr treatment was 48.6% less than the untreated control, and the mean leaf number was 17% greater than all other treatments. This corresponded with higher mean NDVI, where the prothioconazole + benzovindiflupyr treatment had at least 7.5% greater mean NDVI than all other treatments. Treatments with greatest berry yield were not consistent between locations, where treatment mefentrifluconazole + pydiflumetofen had at least 11.2% greater mean yield than all other treatments at one location, and folpet had at least 3% greater mean yield than all other treatments at the other location. For Protocol B, prothioconazole

treatment had consistently low levels of diseased leaves and high leaf number compared to other treatments. The prothioconazole treatment also had consistently high yield between both locations. The results of this study outline the importance of using foliar fungicides to manage the wild blueberry foliar diseases.

2.1 Introduction

Foliar diseases on wild blueberries were once of little concern to growers, but in the past 20 years diseases have become increasingly problematic (Percival & Dawson, 2009). Growers and industry researchers have noted an increase in the presence blueberry foliar diseases leaf rust, Sphaerulina leaf spot (previously Septoria leaf spot) and Valdensia leaf spot, caused by the fungal pathogens *Thekopsora minima*, *Sphaerulina vaccinii* (previously *Septoria* spp.) and *Valdensia heterodoxa*, respectively. Leaf rust is the most common disease, with Sphaerulina leaf spot also being prevalent when environmental factors are conducive and Valdensia leaf spot being of minimal concern (Hildebrand, Renderos & Delbridge, 2016). Sphaerulina leaf spot associated with *S. vaccinii* is the proposed name for this disease (Ali et al., 2021).

Previous work in the early 2000's found that there may not be a single organism responsible for leaf spot symptoms, based on preliminary molecular sequencing for *Septoria* spp. isolated from symptomatic leaves. The original isolate, thought to belong to the Septoria genus, could still be present in wild blueberry fields, but less common than when original testing was completed. The recent molecular identification and classification of *S. vaccinii* (and morphologically similar isolates) found that multiple organisms may cause similar leaf symptoms, but *S. vaccinii* was found to be the most common and it was pursued further. Recently, other preliminary molecular testing found that a small list of

organisms could be responsible for leaf spot symptoms. When the internal transcribed spacer (ITS) region was sequenced for ten cultures grown from leaves with leaf spot symptoms, sequence analysis using basic local alignment search tool (BLAST) showed some cultures had high similarity to *Drechslera biseptata*, *Pestalotiopsis cocculi* or *Pestalotiopsis caudata*, *Phyllostica pyrolae* or *Phyllosticta minima*, *Mycosphaerella nyssicola* or *Mycosphaerella punctiformis* (J. Polashock, personal communication, USDA Chatsworth, NJ, US; A. Cornel unpublished data, Dalhousie University, NS, CA). However, these tentative identifications need further confirmation. Sequencing of additional gene regions such as the elongation factor 1-alpha or the 5.8S gene of the ribosomal rDNA could provide more insight into the exact species. In addition, a thorough Koch's postulates test with these fungal isolates would help determine if they are pathogenic or secondary invaders.

Blueberry leaf rust, caused by *T. minima* is the most common and detrimental foliar disease found on wild blueberries. *T. minima* has a heteroecious disease cycle which uses the eastern hemlock (*Tsuga canadensis*) as an alternate host (Hildebrand et al., 2016). In June aeciospores are windblown from the eastern hemlock and infect wild blueberry leaves (Nguyen, 2019). Once aeciospores have infected the blueberry leaves, small water soaked lesions form on the abaxial leaf surface. From there, red lesions can begin to be distinguished on the adaxial leaf surface. By late July, distinct yellow uredinia pustules, often used for disease identification, form on the abaxial side of the leaves (Polashock et al., 2017). Urediniospores continue to be released from uredinia during the months of August and September which can infect more of the present crop leaves. The polycyclic disease cycle is a key biological feature that makes this disease such a problem for wild blueberry growers.

S. vaccinii is also a prominent foliar pathogen on wild blueberries (Ali et al., 2021). *S. vaccinii* conidia can be identified microscopically as hyaline, smooth, straight, or curved and filiform, being 20 to 110 µm in length. The pycnidia are found on the previous years fallen leaves or stems and begin to release conidia which infect the present year's crop in early June, with peak sporulation time coinciding with the blueberry bloom period (Ali et al., 2021). Symptoms of infected leaves become noticeable in the field by late June with lesions appearing on the abaxial leaf surface as tiny water-soaked lesions, before progressing into lesions on the adaxial leaf surface. Lesions are characterized by a sunken brown center with purple to red margins and usually a yellow necrotic ring surrounding the lesion (Hildebrand et al., 2016). The disease progresses throughout the season with lesions increasing in number and size. Heavily infected leaves are frequently found in the lower part of the crop canopy and lead to premature defoliation (Dawson, 2009).

Blueberry leaf rust and Sphaerulina leaf spot pose a greater problem to wild blueberry growers during the vegetative year of production as compared with the crop year (Dawson, 2009). One reason for this is the timing with which these diseases are found in the field. From August to October leaf disease become increase in incidence and severity. For a vegetative year field this is a problem because August to October also corresponds with the synthesis and allocation of carbohydrates to the floral buds which become next years flowers and berries. On the other hand, in a crop yield field the berries will be harvested during August and September, meaning the crop will not need healthy photosynthetic leaf area moving into the fall months.

Wild blueberry foliar diseases can cause yield losses of up to 100% if unmanaged during the vegetative year of production (Dawson, 2009; Dawson and Percival, 2009). Leaf diseases reduce yields by reducing photosynthetic capacity and causing premature defoliation, thereby the limiting the amount of carbon fixed and allocated for floral bud formation (Dawson, 2009; Garnica et al., 2013; Ojiambo, Scherm, & Brannen, 2006). These diseases have been documented to reduce the net carbon exchange rate by 50% and reduce leaf number by approximately 50% (Dawson, 2009). Premature defoliation reduces the ability for the plants to allocate carbohydrates to floral bud tissues at the end of the growing season, reducing the next year's yields (Roloff, Scherm, & van Iersel, 2004). Similar results have been observed with *Septoria albopunctata* on highbush blueberries. *S. albopunctata* is a prevalent and problematic pathogen found on highbush blueberries in the southern United States, however, has not be documented on wild blueberries. *S. albopunctata* of blueberry plants can reduce net assimilation rate to zero when disease severity is 50%, greatly reducing the number of floral buds produced at the end of the growing season (Ojiambo et al., 2006).

2.1.1 Chemical Control

In the vegetative year, fungicides are typically applied once during the growing season, sometimes twice if disease pressure is high. The first application is made in July to suppress *S. vaccinii* infection in the lower parts of the canopy, as well as to provide protection from *T. minima* (Hildebrand et al., 2016). If high levels of leaf rust are observed in vegetative year fields and weather conditions are conducive for infection, a second fungicide application is made roughly 14 days after the first application. Bravo® ZN (chlorothalonil; Syngenta Canada Inc., Guelph, ON) was once a commonly used fungicide

on wild blueberries for its protective effects of foliar diseases (Percival & Dawson, 2009). Chlorothalonil was used for a number of years independently and provided adequate disease control. When Proline® 480 SC (prothioconazole; Bayer CropScience Inc., Calgary, AB) became registered in Canada, the wild blueberry industry began testing it in screening trials in conjunction with chlorothalonil and observed excellent results (D. Percival, personal communication, Dalhousie University, NS, CA). A tank mix of chlorothalonil and prothioconazole was widely used across the wild blueberry industry for managing foliar diseases. However, wild blueberries were removed from the Bravo® ZN label and shortly after it was deregistered for use in Canada in 2022 (Pest Management Regulatory Agency, 2022). The fungicide received deregistration status due primarily to human health concerns surrounding cancer risks to the general population (Pest Management Regulatory Agency, 2022).

In the last few years, growers have switched to using a tank mix of prothioconazole and benzovindiflupyr as an alternative to chlorothalonil. Prothioconazole (Proline® 480 SC; Bayer CropScience Inc., Calgary, AB) is classified as a demethylation inhibitor (DMI) which inhibits C-14 demethylase during the synthesis of ergosterols in the cell membrane (Parker et al., 2011). Specifically, the N-4 portion of the triazole fungicide compound binds to the C-14 demethylase enzyme, inhibiting its activity (Parker et al., 2013). In the absence of plant pathogens, prothioconazole had significantly higher leaf photosynthesis comparted to untreated leaves (Berdugo et al., 2012). Potential reasoning for extended the duration of green leaf area and delayed senesce is that prothioconazole prevents colonization of saprophytic fungi, which can contribute to plant senescence (Jachmann & Fehrmann, 1989).

Benzovindiflupyr (Aprovia®; Syngenta Canada Inc., Guelph, ON) is classified as a succinate dehydrogenase inhibitor (SDHI) which is absorbed by growing fungal hyphae. It acts to inhibit fungal growth by blocking the activation site of succinate dehydrogenase, effectively stopping the electron transport chain process within the mitochondria of the fungal cell (Kuznetsov et al., 2018). Succinate dehydrogenase is an essential component to all living organisms and it is has been observed that SDHI fungicides can alter succinate dehydrogenase reactions in plants, specifically the transpiration rate and senescence (Kuznetsov et al., 2018). When examined on wheat plants in a controlled environment and field setting, benzovindiflupyr has a dose response effect that reduced transpiration rate in regular conditions and under drought stress. In this case, reduced transpiration rate did not reduce yield (Kuznetsov et al., 2018). Other SDHI fungicides have also been observed to extend green leaf area and leaf senescence, similar to that of prothioconazole and their potential effect on saprophytic fungi (Berdugo et al., 2012). In addition to prothioconazole and benzovindiflupyr there are other fungicides that may become available to blueberry growers in the coming years.

Two fungicides, Cevya® (mefentrifluconazole; BASF Canada Inc., Mississauga, ON), and Miravis® Neo (pydiflumetofen, propiconazole, azoxystrobin; Syngenta Canada Inc, Guelph, ON) were recently registered for use on wild blueberries for management of foliar diseases. Cevya® became registered on wild blueberries in 2023 for use against Botrytis gray mold, Monilinia blight and Septoria leaf spot. Difenoconazole is another DMI fungicide currently registered on other fruit and vegetable crops such as apple, grapes and cucumbers and has a similar range of action against fungal organisms responsible for leaf spot and rust diseases (Pethybridge et al., 2020). Miravis® Neo was recently registered on

wild blueberries and contains a new SHDI fungicide called pydiflumetofen, as well as azoxystrobin and propiconazole. Pydiflumetofen is an effective management tool for leaf spot diseases and rust on a select number of other crops (Pethybridge et al., 2020). Miravis® Neo has not shown excellent effectiveness against leaf diseases (D. Percival, personal communication, Dalhousie University, NS, CA) ; however other products containing pydiflumetofen could be tested on wild blueberries. Folpet (Folpan® 80 WDG; ADAMA Agricultural Solutions Canada Ltd., Winnipeg, MB) is another product that has potential for use on wild blueberries. It has multisite activity on pathogens, similar to that of chlorothalonil, and is approved for use in the EU. Folpet has also shown to be an effective method for controlling various leaf diseases (Bleyer et al., 2020; Sileshi et al, 2014).

Other fungicides that have been discussed within the wild blueberry industry as potentially beneficial for management of the foliar diseases but have not been tested are metconazole, fluoxastrobin and the novel fungicide florylpicoxamid fungicide. Metconazole (Quash®; Valent Canada, Inc., Guelph, ON) belongs to the DMI group of fungicides and is already registered for use on wild blueberries for management of mummy berry disease and effectively manages foliar diseases such as rust and leaf spot on corn (Telenko, Ravellette, & Wise, 2020). Fluoxastrobin (Evito® 480 SC; Arysta LifeScience, Cary, NC) belongs to the quinone outside inhibitors (QoI) fungicide group which interrupt fungal mitochondrial respiration by binding to cytochrome b, effectively stopping the electron transport chain (Ding et al., 2019). It is already registered for use in Canada on strawberries. Fluoxastrobin is a reliable fungicide for the management of multiple fungal species which cause rust on wheat, as well as many fungal species responsible for leaf spot diseases (Martinez-Espinoza et al., 2014; Wise, 2016; Landschoot, 2021). Lastly, the novel

fungicide florylpicoxamid (Corteva Agriscience Canada Company, Calgary, AB) which is considered to belong to a new mode of action which binds to the ubiquinone reduction site on cytochrome b along the mitochondrial electron transport chain, slightly different than QoI fungicides. It is not registered on any crops in Canada however, has been documented to inhibit fungal growth *in vitro* and in a greenhouse setting for a variety of important plant pathogenic species (Yao et al., 2021).

All of the fungicides mentioned above with the exception of folpet have a single mode of action. It is crucial to include multiple modes of action in one fungicide application to increase efficacy and reduce selection for resistance. A fungicide's ability to inhibit fungal growth can be dependent on the growth stage of the pathogen. For example, benzovindiflupyr is highly effective at inhibiting urediniospore germination, whereas the DMI fungicides generally tend to inhibit mycelial growth (see Chapter 3; Siegel, 1981; Slawecki et al., 2002). The use of multiple single site fungicides can reduce the chances for developing resistance (Van Den Bosch et al., 2014). The increased use of single site fungicides, such as those within the SDHI and DMI classes, has led to the decreased efficacy as a result of resistance for some plant pathogens (Miller, Stevenson, & Burpee, 2002). By incorporating two fungicides of different modes of action, the chance of the survival of a resistant mutant is reduced (Van Den Bosch et al., 2014).

2.1.2 UAV Technologies

The premature defoliation of wild blueberries in September and October of the vegetative year is one of the detrimental impacts of foliar pathogens (Nguyen, 2019). In recent years, the use of UAVs (unmanned aerial vehicle) for mapping and remote sensing has become popular for assessing the impact of biotic and abiotic stresses on crops while

reducing the labour requirements to monitor and scout fields (Théau, Gavelle, & Ménard, 2020). Multispectral sensors attached to a UAV can provide accurate recordings of the reflectance of radiation from the visible, red-edge, and near-infrared parts of the electromagnetic spectrum from vegetation below (Olson & Anderson, 2021). The recordings of radiation reflectance from underlying vegetation can then be used to calculate vegetation indices which can differentiate healthy, productive plants from diseased and senesced plants (Assmann et al., 2019). Normalized difference vegetation index (NDVI) has become one of the most popular plant health indices, used to assess disease levels, defoliation and overall crop canopy health in a variety of crops (Olson & Anderson, 2021). The use of UAVs to measure vegetative indices has been used on wild blueberries and has been found to have significant correlations with vegetative nodes, plant height, leaf area index, and yield at different time points during the reproductive stages of production (Anku et al., 2023). Multispectral data have been observed to be strongly correlated with on the ground disease levels from crop scouting (Loladze et al., 2019; Zhang et al., 2018). NDVI values range from 1 to 0, with values close to 1.0 indicating high crop canopy health, whereas values closer to 0 indicate low crop canopy health (Zhang et al., 2018). The use of UAVs to create NDVI maps of wild blueberry fields may provide additional data to support the trends produced from manual stem analyses and foliar disease evaluations in the field.

2.1.3 Objectives

Changes to management practices by growers in response to restrictions, along with the emergence of new fungicides on the market, has led to a need for an updated evaluation of fungicide efficacy for managing wild blueberry foliar diseases. When new products become available on the market, growers require a cost and benefit analysis before adopting

them into their growing practices. With this in mind, the primary objective of this study was to evaluate the impact of both registered fungicides and selected nonregistered fungicides on the percent of leaves infected per stem, leaf number, NDVI, floral bud number, yield components and harvestable berry yield.

2.2 Methods

2.2.1 Experiment Location

Four field trials were conducted in vegetative year commercial fields during the 2021 growing season. Disease efficacy, leaf number and NDVI were collected throughout the 2021 field season, and yield data were collected in 2022. Both commercial field sites have a history of foliar diseases and have neighbouring woodlands near by which can act as an inoculum source for *S. vaccinii*, *T. minima* and *V. heterodoxa*. A commercial field in Kemptown, NS, Canada (45”30°N, 63”07°W) was used for two field trials. This field is comprised of Thom series soil with imperfect drainage, moderate stoniness and of gravelly sandy loam to gravelly silt loam (Webb et al., 1991). This field was managed using commercial standard practices until the spring of the 2021 when the trial was initiated. In the spring of 2021, pre-emergent herbicides foramsulfuron (Option®) and mesotrione (Callisto® 480 SC) were applied at the labelled rate. Granular fertilizer (16-18-10) was applied at a rate of 30 kg·ha^{-1} N, 33.8 kg·ha^{-1} P, and 18.8 kg·ha^{-1} K to sprout field in the spring of 2021. No fungicides for managing leaf diseases were applied to trials except for experimental treatments. In the following spring of 2022, prothioconazole (Proline® 480 SC) fungicide was applied for Monilinia blight control and fluopyram (Luna Tranquility®) fungicide was applied for Botrytis blight control.

A second commercial field in Debert, NS, Canada (45”26°N, 63”27°W) was used as a replicate for the two field trials in Kemptown. Debert and Kemptown sites are located 26 km apart. This field is comprised of Herbert series soil with good drainage, slight stoniness and gravelly loamy sand to gravelly loam over glaciofluvial sand (Webb et al., 1991). This field was managed under regular management practices until the spring of the 2021 when the trial was initiated. In the fall of 2020, herbicides hexazinone (Velpar® DF) and terbacil (Sinbar® WP) were applied at their label rate, with foramsulfuron (Option®) being applied in the spring of 2021. Granular fertilizer (16-18-10) was applied at a rate of 30 kg·ha^{-1} N, 33.8 kg·ha^{-1} P, and 18.8 kg·ha^{-1} K to sprout field in the spring of 2021. No fungicides for managing leaf diseases were applied to trials except for experimental treatments. In the spring of 2022, fungicides prothioconazole (Proline® 480 SC) were applied for Monilinia blight control and fluopyram (Luna Tranquility®) for Botrytis blight control. Fungicides that were applied in the spring of 2022 do not have any impact on the foliar diseases studied during the fall of 2021.

2.2.2 Experimental Design

Treatment plots were marked using plastic plot stakes, with plots being 4 by 4m in size and 2 m wide alleyways between plots. Plastic treatment tags were attached to the plot markers to indicate the treatment (Figure 2.1). The study area consisted of multiple *Vaccinium angustifolium* genotypes and *Vaccinium myrilloides* due to the natural random variation that exists in wild blueberry fields. Protocol A consisted of an untreated control and 10 fungicide treatments with 5 blocks, with one trial at the Kemptown location and another at the Debert location. Protocol B consisted of an untreated control and 6 fungicide treatments with 5 blocks, with one trial at the Kemptown location and another at the Debert

location. Protocol A was used to assess fungicide and active ingredient combinations, while Protocol B aimed to assess single active ingredients that have not been tested on wild blueberries for their management of foliar diseases. Distance to the neighbouring woodland was used as the blocking factor since *T. minima* aeciospore dispersal originates from eastern hemlock trees. Both protocols were setup independently of each other with plots in both protocols arranged as a randomized block design (RBD).

2.2.3 Fungicide Application

Fungicides were applied at the first sign of blueberry leaf rust (*T. minima*) which was on July 20, 2021 at the Kemptown location and July 22, 2021 at the Debert location. Leaf rust was identified by its distinct yellow pustules on the abaxial leaf surface. Fungicides were applied in accordance with their label rates (Table 2.1 and 2.2) with a water volume of 210 $L \cdot ha^{-1}$. Fungicides in their commercially available form were mixed with tap water (pH of 6.85) in 2 L spray bottles to their specified concentrations and shaken in the bottles to mix effectively. When multiple fungicides were used in one treatment, they were mixed in the same container and applied together. Application was made using a Bellspray Inc. Model GS carbon dioxide pressurized hand sprayer 30 cm above the crop canopy at 220 kPa with a 2 m wide spray pattern fit with four Teejet Visiflow 80-02VS nozzles (Figure 2.1). The nozzle discharge rate was 10 $mL \cdot s^{-1}$ and the application ground speed was 1 $m \cdot s^{-1}$. To ensure accurate application rate to the plots, the amount of liquid emitted from the nozzles was measured and used to calculate walking speed. A metronome was used to maintain constant walking speed. Two side by side passes were made to evenly cover the entire 4 by 4 m plot.

Figure 2.1. (A) Plastic stakes used to mark 4 x 4 m treatment plots in commercial wild blueberry fields in Debert and Kemptown, NS, CA, (B) fungicide treatment application using a 2 m wide boom and carbon dioxide as the propellant.

Table 2.1 Fungicide treatments, active ingredients, year registered and application rate used in Protocol A at the Kemptown and Debert locations. All fungicides were applied in accordance with their label rates at a water volume of 210 L·ha^{-1}. Year registered refers to the year these fungicides were registered on wild blueberries in Canada.

Treatment	Active Ingredient	Year Registered[z]	Rate (L·ha^{-1})
Untreated control			
Cevya® [Y]	Mefentrifluconazole	2023	0.25
Cevya®[Y] + Sercadis® [Y]	Mefentrifluconazole+flupyroxad	2023/2016	0.25 + 0.6
Cevya® + Miravis® Bold [X]	Mefentrifluconazole+pydiflumetofen	2023/NR	0.25 + 1.0
Miravis® Bold [X]	Pydiflumentofen	NR	1.0
Miravis® Duo [X]	Pydiflumetofen+difenoconazole	NR	1.0
Proline® 480 SC [W]	Prothioconazole	2011	0.4
Propulse® [W]	Prothioconazole+fluopyram	2019	1.0
Proline® 480 SC [W] + Aprovia® [X]	Prothioconazole+benzovindiflupyr	2011/2016	0.4 + 0.75
Proline® 480 SC [W] + Miravis® Bold [X]	Prothioconazole+pydiflumetofen	2011/NR	0.4 + 1.0
Folpan® 80 WDG [V]	Folpet	NR	2.5 kg·ha^{-1}

[Z] NR=not registered
[Y] manufactured by BASF Canada Inc.
[X] manufactured by Syngenta Canada Inc.
[W] manufactured by Bayer CropScience Inc.
[V] manufactured by ADAMA Agricultural Solutions Canada Ltd.

Table 2.2 Fungicide treatments, active ingredients and application rate used in Protocol B at the Kemptown and Debert locations. All fungicides were applied in accordance with their label rates at a water volume of 210 L·ha^{-1}. Year registered refers to the year these fungicides were registered on wild blueberries in Canada.

Treatment	Active Ingredient	Year Registered[z]	Rate (L·ha^{-1})
Untreated control			
Cevya® [Y]	Mefentrifluconazole	2023	0.25
Proline® 480 SC [W]	Prothioconazole	2011	0.4
Quash® [U]	Metconazole	2015	0.2
Evito® 480 SC [T]	Fluoxastrobin	NR	0.25
Adavelt®	Florylpicoxamid	NR	1
Folpan® [V]	Folpet	NR	2.5 kg·ha^{-1}

[Z] NR=not registered
[Y] manufactured by BASF Canada Inc.
[W] manufactured by Bayer CropScience Inc.
[V] manufactured by ADAMA Agricultural Solutions Canada Ltd.
[U] manufactured by Valent Canada Inc.
[T] manufactured by Arysta LifeScience
[S] manufactured by Corteva Agriscience Canada Company

2.2.4 Assessments and Statistical Analysis

Number of diseased leaves per stem for each disease (Valdensia leaf spot (Figure 2.2A), Sphaerulina leaf spot (Figure 2.2B), and blueberry leaf rust (Figure 2.2C)) and leaf number per stem were measured 30 and 60 days after application (DAA) (±5 days). The number of diseased leaves per stem was converted into a percentage since heavily infected plants tend to lose their leaves and, the number of infected leaves per stem does not accurately describe the amount of disease. Floral bud number per stem was measured 120 days after application (±5 days). Assessments were made by randomly selecting 15 stems along a 4-m linear transect within each plot. The stem samples were cut at the soil surface and placed in plastic bags where they were taken to the laboratory for analysis.

On October 5th, 2021 (77 DAA) the normalized difference vegetation index (NDVI) was measured using a DJI Matrice 600 Pro (SZ DJI Technology Co., Ltd; Shenzhen, China)

UAV flown at a height of 30 m fit with a 5-band MicaSense RedEdge™ 3 (MicaSense, Inc.; Seattle, US) multispectral sensor to collect reflected light at the wavelengths; blue (475), green (560), red (668), red edge (717) and near-infrared (NIR) (840). NDVI was calculated using the red and NIR reflectance (NDVI = NIR – red / NIR + red). Multispectral data was analysed in PrecisionHawk's PrecisionAnalytics Agriculture (PrecisionHawk; Toronto, CA) (now called Solvi AB, Gothenburg, Sweden) web-based portal. Using PrecisionAnalytics Agriculture, orthomosaic imagery of the plots was generated and used to calculate mean NDVI for each plot. Berries were harvested on August 18, 2022 with a lowbush blueberry hand rake from four randomly selected 1 m^2 quadrats within each plot. The mass of berries from each quadrat was measured using an Avery Mettler PE 6000 digital balance (Figure 2.3).

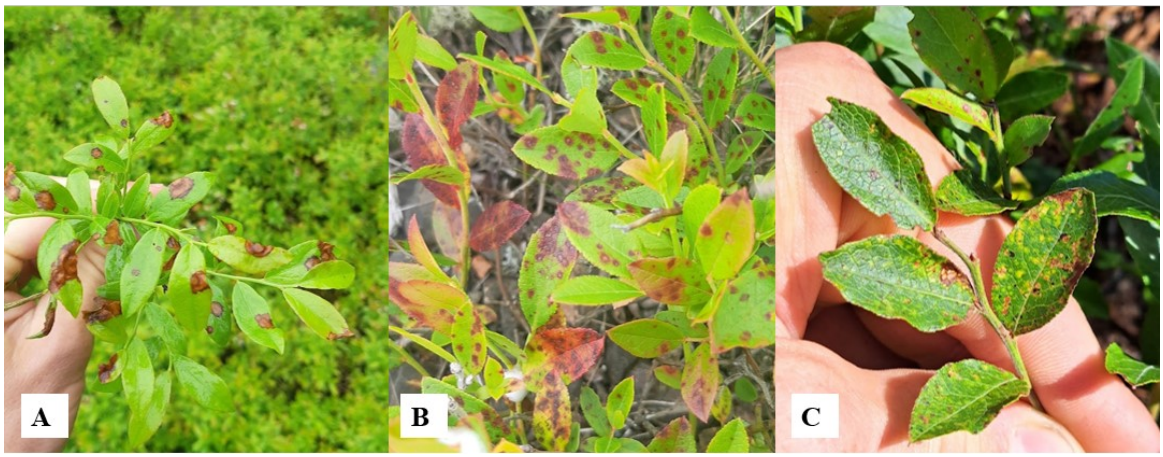

Figure 2.2 (A) Mature lesions of Valdensia leaf spot in June, (B) mature lesions of Sphaerulina leaf spot in late July, (C) Immature lesions of blueberry leaf rust in late July. Diseases were observed in commercial wild blueberry fields in Nova Scotia, CA.

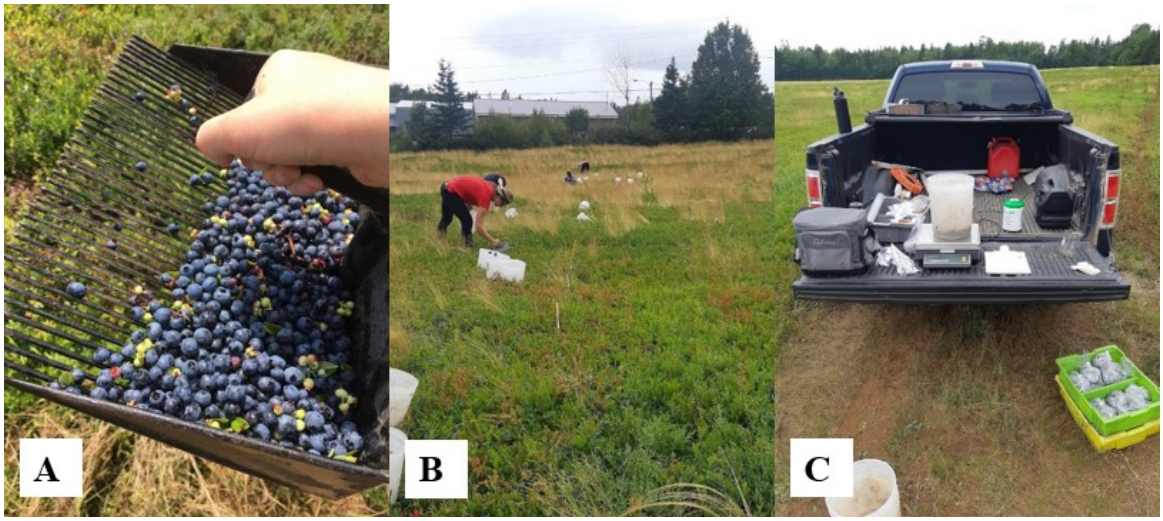

Figure 2.3. (A) Using a lowbush blueberry hand rake to harvest treatment plots, (B) research interns assisting with blueberry harvest, (C) measuring the mass of blueberries harvested from each quadrat during blueberry harvest in Debert, NS.

Before conducting the ANOVA for response variables, the normality of the error terms, constant variance and independence were verified. Using Minitab (version 19.1.1) the residuals were plotted in a normal probability plot (NPP) of residuals, and residuals versus fitted values. If the error terms were not normal, the response variable was transformed by Y^2, $Y^{1/2}$, $Y^{1/3}$, ln(Y), log(Y), $Y^{-1/2}$ or Y^{-1}, where Y is the response variable. Transformations were completed in Minitab (version 19.1.1). Transformations were done in this order, with the residuals recalculated and normality of error terms checked after each transformation. Response variables that were transformed to satisfy the assumptions are listed in Table 2.3. Mean NDVI per plot did not have to be transformed for either location.

Once normalized, response variables were analysed using PROC GLIMMIX procedure of SAS (version 9.4, SAS institute, Inc., Cary, NC). Location by treatment interactions were assessed at $\alpha = 0.05$. Each assessment date and protocol were analysed separately, and location was analysed separately when a treatment by location interaction was found. Fisher's Least Significance Differences (LSD) was used for multiple means comparisons at $\alpha = 0.05$.

Table 2.3 Response variables percent leaves infected, leaf number, floral bud number, fruit set and harvestable berry yield that required transformations to satisfy the normality of the error terms and constant variance prior to conducting the ANOVA. Where no transformation is shown, no transformation was required to satisfy the assumptions.

			Transformation Performed	
Response variable	**Location**	**DAA**	**Protocol A**	**Protocol B**
Leaf number	Debert	30	$Y^{1/2}$	
	Debert	60	$Y^{1/2}$	$Y^{1/2}$
	Kemptown	30	$Y^{1/2}$	ln(Y)
	Kemptown	60		
Percent leaves with Sphaerulina leaf spot	Debert	30	$Y^{1/2}$	
	Debert	60	$Y^{1/2}$	Y^{2}
	Kemptown	30	$Y^{1/2}$	
	Kemptown	60		Y^{2}
Percent leaves with leaf rust	Debert	30	$Y^{1/2}$	
	Debert	60		Y^{2}
	Kemptown	30	$Y^{1/2}$	
	Kemptown	60		Y^{2}
Sum percent leaves infected	Debert	30	$Y^{1/2}$	
	Debert	60		Y^{2}
	Kemptown	30	$Y^{1/2}$	
	Kemptown	60		Y^{2}
Floral bud number	Debert	120		$Y^{1/2}$
	Kemptown	120		ln(Y)
Fruit set	Debert		Y^{2}	$Y^{1/2}$
	Kemptown		$Y^{1/2}$	$Y^{1/2}$
Harvestable Yield	Debert			
	Kemptown		$Y^{1/2}$	

2.3 Results

2.3.1 Protocol A

Disease, Leaf Number, NDVI, Floral Bud Number

Thirty days after application, treatment x location interactions ($P<0.01$) were observed for leaf number and percent leaves diseased assessments, so each location was assessed independently. At the Kemptown location, all treatments with prothioconazole had at least 62.6% lower disease than the untreated. Leaf rust was more prevalent than Sphaerulina and Valdensia leaf spot at the Kemptown location 30 DAA (Table 2.4).

Prothioconazole with benzovindiflupyr had significantly greater leaf number than all other treatments, being 24.6% greater than in the untreated plots (Table 2.4).

At the Debert location 30 DAA, results were contrary to what was seen in Kemptown. Mefentrifluconazole with pydiflumetofen had 90.4% less disease than the untreated (Table 2.5). All fungicide treatments had significantly less disease than the untreated control, with Sphaerulina leaf spot appearing more frequently in all plots than leaf rust (Table 2.5). To go along with the disease evaluation, mefentrifluconazole with pydiflumetofen had the greatest leaf number being 9.6% greater than the untreated (Table 2.5).

Sixty days after application, treatment x location interactions ($P<0.01$) were observed for leaf number and percent diseased leaves assessments, so each location was assessed independently. At the Kemptown location, prothioconazole with benzovindiflupyr had at least 48.6% less diseased leaves per stem compared to the untreated (Table 2.4), and at the Debert site, treatments prothioconazole with benzovindiflupyr, prothioconazole with pydiflumetofen and folpet had at least 75% fewer diseased leaves per stem compared to the untreated (Table 2.5). At both locations, prothioconazole with benzovindiflupyr had at least 17% greater leaf number than all other treatments and at least 44.5% more than the untreated control (Table 2.4 and 2.5). Variation in the efficacy of folpet was noted between locations, with it providing a high level of disease control at the Debert location compared to the Kemptown location (Table 2.4 and 2.5).

Table 2.4 The effect of fungicide treatments on the mean leaf number per stem, mean percent diseased leaves per stem and sum of percent leaves infected per stem for the two diseases assessed at the Kemptown location (Protocol A).

	30 DAA				60 DAA			
		Percent diseased leaves (%)[1]				Percent diseased leaves (%)[1]		
Treatment	Leaf number	Rust	Sphaerulina	Sum	Leaf number	Rust	Sphaerulina	Sum
Untreated control	19.1bcd	20.6a	1.78bc	22.4a	4.23ef	54.7bcd	2.56ab	57.2bcd
Mefentrifluconazole	21.6b	11.2b	2.74ab	14.0b	3.56f	38.4ef	0.69bc	39.1ef
Mefentrifluconazole + fluopyroxad	18.4cd	20.8cd	3.79ab	24.6a	8ef	50.5bcde	0.33c	50.8bcde
Mefentrifluconazole + pydiflumetofen	17.0d	17.4a	0.66c	18ab	3.81ef	48.3de	0.00c	48.3de
Pydiflumentofen	18.1cd	6.44c	0.62c	7.06c	5.88ef	49.2cde	0.29c	49.5cde
Pydiflumetofen + difenoconazole	19.1bcd	10.0b	5.22a	15.2b	9.28cd	58.6bcd	2.81a	61.4abcd
Prothioconazole	19.8bc	3.19cd	0.73c	3.92cd	12.2bc	72.3a	0.24c	72.6a
Prothioconazole + fluopyram	18.9bcd	4.46c	1.42bc	5.88c	6.12e	49.5cde	0.78bc	50.3bcde
Prothioconazole + benzovindiflupyr	25.3a	0.00e	1.60d	1.60d	19.5a	28.3f	0.30c	28.6f
Prothioconazole + pydiflumetofen	19.3bcd	7.70c	2.00bc	9.70c	11.5bc	62.2abc	1.29abc	63.5abc
Folpet	21.3b	1.04de	0.63c	1.67d	8.95d	63.4ab	0.00c	63.4ab
ANOVA results[2]	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P=0.03	P<0.001

[1] Percent of leaves per stem infected with blueberry leaf rust or Sphaerulina leaf spot and the sum of the two diseases.
[2]ANOVA results refer to treatment effects that were not significant (NS) or significant at P<0.05. Mean comparison was completed using LSD test ($\alpha = 0.05$). Means in a column with the same letter are not significantly different from each other.

Table 2.5 The effect of fungicide treatments on the mean leaf number per stem, mean percent diseased leaves per stem and sum of percent leaves infected per stem for the two diseases assessed at the Debert location (Protocol A).

	30 DAA				60 DAA			
		Percent diseased leaves (%)[1]				Percent diseased leaves (%)[1]		
Treatment	Leaf number	Rust	Sphaerulina	Sum	Leaf number	Rust	Sphaerulina	Sum
Untreated control	19.8abc	2.90b	8.2a	11.1a	13.3e	76.0a	3.59a	78.1a
Mefentrifluconazole	20.8bcd	1.30cd	3.0bc	4.28bcd	19bc	28.3e	3.07ab	31.4de
Mefentrifluconazole + fluopyroxad	20.8ab	2.47bc	2.3bcde	4.81bcd	16.3d	52.8b	0.10d	52.8b
Mefentrifluconazole + pydiflumetofen	21.9a	0.34d	0.7e	1.07f	18bcd	38.4cd	0.25d	38.7cd
Pydiflumentofen	18.2cd	1.64bcd	1.7bcde	3.32cde	18.9bc	46.5bc	1.23bcd	47.8bc
Pydiflumetofen + difenoconazole	17.8d	2.21c	3.9b	6.13bc	17.4cd	40.6bc	0.72cd	41.3cd
Prothioconazole	20abc	4.82a	2.3bcde	7.14b	18.6bc	37.0de	2.30abc	38.7cde
Prothioconazole + fluopyram	17.2d	0.29d	1.4cde	1.70ef	19.9b	26.8de	0.63cd	27.5ef
Prothioconazole + benzovindiflupyr	21.6a	0.07d	1.7bcde	1.74ef	24.2a	10.4f	0.84cd	11.3gh
Prothioconazole + pydiflumetofen	17.5cd	0.38d	1.1de	1.43ef	18.1bcd	18.7f	0.17d	18.9fg
Folpet	18.3bcd	0.08d	4.5bcd	4.54cde	19.5bc	7.10f	0.63cd	7.73h
ANOVA results[2]	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

[1] Percent of leaves per stem infected with blueberry leaf rust or Sphaerulina leaf spot and the sum of the two diseases.

[2]ANOVA results refer to treatment effects that were not significant (NS) or significant at P<0.05. Mean comparison was completed using LSD test ($\alpha = 0.05$). Means in a column with the same letter are not significantly different from each other.

On October 5th, 2021, NDVI was measured at both locations. Treatment x location interaction was observed for mean NDVI ($P<0.01$), so each location was assessed independently. Mean NDVI for prothioconazole with benzovindiflupyr treatment plots was 13.8% and 62% greater than the untreated at the Kemptown (Table 2.6) and Debert (Table 2.7) locations, respectively. This is consistent with the disease and leaf number evaluations made 60 DAA.

On November 20th, 2021 (120 DAA) floral bud number was assessed at both locations. Treatment x location interaction was observed for mean floral bud number per stem ($p<0.01$), so each location was assessed independently. The treatment mefentrifluconazole with pydiflumetofen had the greatest floral bud number per stem at both locations, being 29.9% to 51.8% greater than the untreated plots (Table 2.6 and 2.7). Despite mefentrifluconazole with pydiflumetofen not having particularly high leaf number or low amounts of diseased leaves per stem, it was seen to have the greatest floral bud number, an indicator of potentially greater yield compared to other treatments (Table 2.6 and 2.7).

Harvestable Berry Yield

For fruit set data and harvestable berry yield, there was a treatment x location interaction ($P<0.01$), so each location was assessed independently. Average yield at Kemptown was 513.2 g/m^2 (Table 2.6), while it was 688 g/m^2 at Debert (Table 2.7). At the Kemptown location, mefentrifluconazole with pydiflumetofen had the greatest yield, being 53% greater than the untreated control and at least 11.2% greater than all other treatments (Table 2.6). This is consistent with the floral bud numbers that were evaluated in November of 2021. At the Kemptown site, despite the low disease present for the prothioconazole

with benzovindiflupyr treatment, it only had 25.2% greater yield than the untreated, and at Debert site the untreated control actually had 21% greater yield than prothioconazole with benzovindiflupyr (Table 2.6).

Table 2.6 The effect of fungicide treatment on mean NDVI per plot, mean floral bud number per stem, mean fruit set per stem and mean harvestable berry yield (g/m^2) assessed at the Kemptown location (Protocol A).

Treatment	NDVI	Floral bud number	Fruit Set	Harvestable berry yield (g/m2)
Untreated control	0.69bc	2.24c	4.69	371.2de
Mefentrifluconazole	0.71bc	2.81bc	4.40	485.3cd
Mefentrifluconazole + flupyroxad	0.67c	3.84abc	3.98	505.9cde
Mefentrifluconazole + pydiflumetofen	0.68bc	4.65a	5.63	789.2a
Pydiflumentofen	0.71bc	3.36abc	5.00	352.2de
Pydiflumetofen + difenoconazole	0.74ab	2.61bc	6.30	314.4e
Prothioconazole	0.71bc	3.44abc	5.96	461.2cde
Prothioconazole + fluopyram	0.70bc	4.05ab	5.21	701.8ab
Prothioconazole + benzovindiflupyr	0.80ab	4.25ab	5.60	496.2cd
Prothioconazole + pydiflumetofen	0.74ab	3.55abc	5.88	619.0abc
Folpet	0.74ab	3.63abc	5.94	548.8bc
ANOVA results[2]	P=0.003	P<0.001	NS	P<0.001

[2]ANOVA results refer to treatment effects that were not significant (NS) or significant at $P<0.05$. Mean comparison was completed using LSD test ($\alpha = 0.05$). Means in a column with the same letter are not significantly different from each other.

Table 2.7 The effect of fungicide treatment on mean NDVI per plot, mean floral bud number per stem, mean fruit set per stem and mean harvestable berry yield (g/m^2) assessed at the Debert location (Protocol A).

Treatment	NDVI	Floral Bud Number	Fruit Set	Harvestable berry yield (g/m2)
Untreated control	0.19d	4.04abc	9.10	789.3ab
Mefentrifluconazole	0.26cd	3.56bc	6.88	650.3bcd
Mefentrifluconazole + flupyroxad	0.43a	4.76abc	6.58	623.1cd
Mefentrifluconazole + pydiflumetofen	0.27cd	5.76a	9.32	604.4cd
Pydiflumentofen	0.29bcd	3.17c	6.70	530.0d
Pydiflumetofen + difenoconazole	0.38abc	4.17abc	7.17	710.7abc
Prothioconazole	0.37abc	4.65abc	10.6	750.8abc
Prothioconazole + fluopyram	0.41ab	5.05ab	8.51	717.3abc
Prothioconazole + benzovindiflupyr	0.50a	4.96ab	7.98	620.7cd
Prothioconazole + pydiflumetofen	0.44a	5.19ab	9.44	758.7abc
Folpet	0.41ab	5.07ab	7.07	813.2a
ANOVA results[2]	P<0.001	P<0.001	NS	P=0.005

[2]ANOVA results refer to treatment effects that were not significant (NS) or significant at P<0.05. Mean comparison was completed using LSD test ($\alpha = 0.05$). Means in a column with the same letter are not significantly different from each other.

2.3.2 Protocol B

Disease, Leaf Number, NDVI, Floral Bud Number

Thirty days after application, treatment x location interactions (P<0.01) were seen for percent diseased leaves and leaf number so each location was assessed independently. At both locations, the prothioconazole treatment had considerably less diseased leaves per stem than the majority of other treatments with between 61.5 and 55.2% fewer disease than the untreated (Table 2.8 and 2.9). Diseased leaves per stem did not appear to be a consistent predictor of leaf number per stem across all treatments, however fluoxastrobin had the greatest leaf number at both locations and had 72.0 % less disease than the untreated at the Debert location (Table 2.9).

Sixty days after application, treatment x location interactions ($P<0.01$) were seen for percent diseased leaves and leaf number assessments, so each location was assessed independently. At the Kemptown location, metconazole maintained low percent diseased leaves per stem being at least 22.8% lower than all other treatments. This did not translate to increased leaf number, with prothioconazole having at least 17.9% greater leaf number than all other treatments (Table 2.8). At the Debert location, prothioconazole and fluoxastrobin had at least 25.6% less disease than the untreated. For the prothioconazole treatment, this did translate to significantly greater leaf number than all other treatments (Table 2.9).

Table 2.8 The effect of fungicide treatments on the mean leaf number per stem, mean percent diseased leaves per stem and sum of percent leaves infected per stem for the two diseases assessed at the Kemptown location (Protocol B).

	30 DAA				60 DAA			
		Percent diseased leaves (%)[1]				Percent diseased leaves (%)[1]		
Treatment	Leaf number	Rust	Sphaerulina	Sum	Leaf number	Rust	Sphaerulina	Sum
Untreated	16.5b	22.7a	4.44ab	27.2a	2.59c	30.3	1.33	31.7bc
Mefentrifluconazole	20.7a	18.5ab	2.83b	21.3a	4.20bc	47.3	1.39	48.7a
Prothioconazole	17.7ab	4.57c	7.63a	12.2bc	6.99a	44.8	1.41	46.2a
Metconazole	18.4b	7.94c	2.57b	10.5c	4.32bc	23.4	1.11	24.6c
Fluoxastrobin	21.5a	20.1ab	2.63b	22.8a	4.72b	38.3	0.21	38.6ab
Florylpicoxamid	17.1b	16.4ab	6.11a	22.5a	6.96a	49.4	0.48	49.8a
Folpet	17.2b	15.3b	4.43b	19.7ab	3.67bc	18.3	0	18.3c
ANOVA results[2]	P<0.001	P<0.001	P=0.013	P=0.001	P<0.001	P<0.001	NS	P<0.001

[1] Percent of leaves per stem infected with blueberry leaf rust or Sphaerulina leaf spot and the sum of the two diseases.

[2]ANOVA results refer to treatment effects that were not significant (NS) or significant at P<0.05. Mean comparison was completed using LSD test ($\alpha = 0.05$). Means in a column with the same letter are not significantly different from each other.

Table 2.9 The effect of fungicide treatments on the mean leaf number per stem, mean percent diseased leaves per stem and sum of percent leaves infected per stem for the two diseases assessed at the Debert location (Protocol B).

	30 DAA				60 DAA			
		Percent diseased leaves (%)[1]				Percent diseased leaves (%)[1]		
Treatment	Leaf number	Rust	Sphaerulina	Sum	Leaf number	Rust	Sphaerulina	Sum
Untreated	17.3ab	12.6a	1.32bc	13.9ab	12.0c	80.2ab	0.32	80.5b
Mefentrifluconazole	15.5b	8.34b	0.79bc	9.13cd	9.39c	81.1ab	0.60	81.7ab
Prothioconazole	17.5ab	3.84cd	1.52bc	5.35de	17.4a	42.2d	0.74	42.9d
Metconazole	15.3b	3.79cd	3.47a	7.26cde	13.4b	77.5b	1.31	78.8b
Fluoxastrobin	19.0a	3.31d	0.58c	3.89e	14.3b	59.4c	0.16	59.5c
Florylpicoxamid	16.7b	15.9a	1.69abc	17.6a	10.3c	88.7ab	0.54	89.3a
Folpet	15.9b	7.47bc	2.55ab	10bc	10.8c	78.1b	1.33	79.4b
ANOVA results[2]	P=0.019	P<0.001	P=0.029	P<0.001	P<0.001	P<0.001	NS	P<0.001

[1] Percent of leaves per stem infected with blueberry leaf rust or Sphaerulina leaf spot and the sum of the two diseases.

[2]ANOVA results refer to treatment effects that were not significant (NS) or significant at P<0.05. Mean comparison was completed using LSD test ($\alpha = 0.05$). Means in a column with the same letter are not significantly different from each other.

The mean NDVI per plot showed no treatment by location interaction (P=0.24) so both locations were assessed together. No significant differences between treatments were observed. Kemptown had greater overall NDVI compared to at the Debert location, despite leaf number overall being greater at the Debert location (Table 2.10 and 2.11). At the Debert location prothioconazole had 68.3% greater mean NDVI compared to the untreated and 48.8% greater mean NDVI compared to all other treatments (Table 2.11).

At approximately 120 DAA floral bud number was measured. There was a treatment x location interaction (P<0.01), so each location was assessed independently. At the Kemptown location, the leaf number and percent of leaves with disease (Table 2.8) did not necessarily correspond to floral bud number (Table 2.10). Despite having low leaf number 60 DAA, the untreated had 22.6% greater floral bud number than all other treatments. This was not seen at the Debert location, where prothioconazole had the greatest leaf number 60 DAA, and also had the greatest floral bud number, with 26.1% more floral bud number than the untreated control (Table 2.11).

Harvestable Berry Yield

There was a treatment x location interaction (P<0.01), so each location was assessed independently. Overall harvestable berry yield was very similar between locations with Kemptown having 590.8 g/m^2 (Table 2.10) and Debert having 584.2 g/m^2 (Table 2.11). Prothioconazole had consistently high yields at both locations, being at least between 20.3 and 43.4% greater compared to the untreated.

Table 2.10 The effect of fungicide treatment on mean NDVI per plot, mean floral bud number per stem and mean harvestable berry yield (g/m^2) assessed at the Kemptown location (Protocol B).

Treatment	NDVI	Floral Bud Number	Fruit Set	Harvestable berry yield (g/m2)
Untreated	0.63	4.11	13.5	436.4
Mefentrifluconazole	0.68	4.97	12.2	497.0
Prothioconazole	0.66	5.56	14.5	771.1
Metconazole	0.64	5.23	13.4	676.5
Fluoxastrobin	0.67	4.55	11.6	608.4
Florylpicoxamid	0.68	4.40	10.6	593.4
Folpet	0.66	5.17	12.5	553.1
ANOVA results[2]	NS	NS	NS	NS

[2]ANOVA results refer to treatment effects that were not significant (NS) or significant at $P<0.05$. Mean comparison was completed using LSD test ($\alpha = 0.05$). Means in a column with the same letter are not significantly different from each other.

Table 2.11 The effect of fungicide treatment on mean NDVI per plot, mean floral bud number per stem and mean harvestable berry yield (g/m^2) assessed at the Debert location (Protocol B).

Treatment	NDVI	Floral Bud Number	Fruit Set	Harvestable berry yield (g/m2)
Untreated	0.13	5.98a	7.0	544.2bcd
Mefentrifluconazole	0.13	3.10c	6.6	481.1d
Prothioconazole	0.41	4.63ab	8.3	682.4ab
Metconazole	0.18	3.90bc	6.4	530.7cd
Fluoxastrobin	0.14	3.87bc	8.9	643.3abc
Florylpicoxamid	0.10	3.03c	5.7	502.3cd
Folpet	0.21	3.75bc	8.5	705.6a
ANOVA results[2]	NS	P=0.001	NS	P=0.006

[2]ANOVA results refer to treatment effects that were not significant (NS) or significant at $P<0.05$. Mean comparison was completed using LSD test ($\alpha = 0.05$). Means in a column with the same letter are not significantly different from each other.

2.4 Discussion

Weather station data collected during the 2021 season from Debert and a blueberry field near Kemptown (Mt. Thom; 10 km from Kemptown), provided insight into potential infection periods based on temperature, relative humidity and leaf wetness data (Figure A-1 and A-2; Table A-1 and A-2). The weather station data were compared to previous studies

that have examined the impact of temperature, rainfall, and humidity on *T. minima* and *S. vaccinii* growth (Abbasi et al., 2022; Gupta et al., 2017; Pfister et al., 2004). Gupta et al. (2017) assessed the effect of abiotic conditions on the severity of stripe rust of wheat. They observed that minimum temperature, maximum temperature and rainfall explained 91% of the variation in response variable. Pfister et al. (2004) found that the optimal temperature ranges for *T. minima* urediniospore germination, incubation period and uredinia production were 19-23 ℃, 20-26 ℃ and 17.5-22 ℃, respectively. Leaf wetness can significantly impact leaf diseases, for soybean rust in the United States it has been documented that a leaf wetness duration of >18 hrs results in significantly greater disease incidence and severity (Narváez et al., 2010). For *S. vaccinii*, Abbasi et al. (2022) observed that the optimal temperature for conidia germination was 15-25 ℃ and optimal humidity for infection was >90% RH. Chungu, Gilbert, & Townley-Smith (2001) found that longer periods of leaf wetness increased *Septoria tritici* blotch development and that leaf wetness of at least 48 hrs resulted in high infection. By comparing the result of these studies to the conditions observed from June to September 2021, high risk or moderate risk infection periods were grouped together for *S. vaccinii* and *T. minima*.

The weather data collected at Mt. Thom may be quite different than the conditions that were observed in Kemptown fields due to the location of the weather station in the Mt. Thom field. The trial in Kemptown was situated alongside a treeline and had much less airflow compared to the Mt. Thom field. This variation should be considered when assessing the Mt. Thom weather data and comparing it to Debert weather station data. The Mt. Thom weather station data indicated there were three high risk potential infection periods in June. In July there were four moderate risk potential infection periods, and one

high risk potential infection period. In August there were two moderate risk potential infection periods and two high risk potential infection periods on August 4 and August 18, 2021 (Figure A-1, Table A-1). September data were not collected at the Mt. Thom field.

The weather station set up in Debert was situated close to a neighbouring forest and the equipment shed. This would influence the airflow near the station, and this should be considered when examining the leaf wetness and humidity data in comparison to the Mt. Thom data. According to the weather station data collected in Debert during the 2021 season, conditions were consistently conducive for infection across June, July, August and September. In June, there were two moderate and three high risk potential infection periods. In July, there were nine moderate risk potential infection periods and five high risk potential infection periods. In August there were eleven moderate risk potential infection periods and eight high risk potential infections periods. September conditions were comparable to August's, with seven moderate risk potential infection periods and six high risk potential infection periods (Figure A-2, Table A-2). The Debert weather station data shows a greater number of infection periods compared to the Mt. Thom weather station, however; the difference in field placement of the stations should be considered.

The weather station data can be used to explain some of the disease trends observed during the August and September disease assessments. The number of diseased leaves per stem increased from the August to September assessment dates. During the months of August and September the conditions observed in Debert were highly conducive for infection. Periods of extended leaf wetness (ie. >12 hrs), high relative humidity (ie. >85%) and temperatures between 15 and 25 °C provided excellent conditions for infection (Table A-1, Table A-2). Conditions of high humidity and extended leaf wetness are especially

enabling for fungal pathogens because these conditions allow spores to germinate more successfully, and aids in the production of spores. Without high humidity and free water, some fungi are unable to produce spores (Agrios, 2005). Temperature can also influence fungal infection and spore production as it can influence the metabolic rate of fungi (Talley, Coley, & Kursar, 2002). Pfister et al., (2004) studied the effect of temperature on *T. minima* uredinia, the distinct yellow pustules on the abaxial leaf surface, production on *Rhododendron* leaves. They found that uredinia production did not vary significantly between 15, 20 and 25 ℃, but was significantly reduced at 30 ℃. The conditions for peak uredinia production correspond with the field conditions and disease observations in the present study.

In addition to conducive conditions for infection, the increase in rust presence from 30 to 60 DAA was possibly due to its polycyclic disease cycle and its ability to release urediniospores multiple times after initial infection has occurred (Hildebrand et al., 2016). Researchers from the University of Maine analysed weather data and *T. minima* spore dispersal patterns during the 2014, 2015 and 2017 growing seasons (Nguyen, 2019). They found that in commercial wild blueberry fields, the leaf wetness duration increased as the growing season progressed, similar to conditions observed in the present study. They also found that based on manual spore counting and quantitative polymerase chain reaction (qPCR) spore detection, *T. minima* aeciospores were detected in the air, migrating from their alternate host to blueberries in late June to early July. The initial infection by aeciospores does not necessarily cause abundant disease symptoms, that is typically caused by the repeated release and infection of the urediniospores. Urediniospores were observed to be present in the air surrounding blueberry fields from late August until mid-October.

Peak urediniospore dispersal was found to be in mid-September, with a sharp increase in symptomatic leaves 14 days after (Nguyen, 2019). These results reenforce the findings of increased leaf rust in September compared to August found in the present study.

For Protocols A and B, the number of diseased leaves per stem was found to be higher and the leaf number per stem was found to be lower at the Kemptown location compared to Debert. This was also observed for harvestable berry yield which was lower at Kemptown than Debert. These results contradict the leaf wetness, temperature and relative humidity data that was observed at the two locations, where the conditions in Debert appear to have more potential infection periods than the Mt. Thom data. As mentioned, the placement of the weather station in the fields was very different and is a plausible reason for the differences in observed weather conditions (Figure A-1, Figure A-2).

NDVI measured using multispectral sensors attached to UAVs have been observed as having a high correlation with on the ground disease levels from crop scouting and can remove the labour intensive job of scouting (Loladze et al., 2019; Zhang et al., 2018). With this is mind, the relationship of NDVI to other physiological or pathological assessments could be determined using correlations. NDVI has been observed as having a weak correlation with floral bud number, and berry yield and moderate correlation with plant height and leaf number, However, these relationships can be highly variable between locations (Kenneth Anku, MSc, personal communication, Dalhousie University, NS, CA; Barai et al.,2021). Similarly, brief correlation analysis was done for this trial to assess the relationship between NDVI and plant height, leaf number, disease, floral bud number and yield and weak to moderate correlations were seen.

Despite the Kemptown location having higher disease pressure and greater defoliation by October 5th, the mean NDVI across all treatments was still greater at the Kemptown (Table 2.6) compared to Debert (Table 2.7). Some potential reasons for this may have been due to the Kemptown plots having a greater presence of weeds compared to Debert. Weeds were not able to be removed from the UAV imaging or NDVI calculations. Red sorrel (*Rumex acetosella* L.) and fescue (*Festuca* spp.) were apparent is many plots, with red sorrel maintaining green leaves into November and fescue senescing in late September, potentially influencing the October 5th imagery (D'Appollonio, 2018ab). Similar results were observed for Protocol B at the Kemptown location.

Regarding the effect of fungicide treatment on percent leaves infected, leaf number per stem, NDVI, floral bud number and yield, treatment by location interactions were observed. One potential reason for this could be the variation in the efficacy of folpet which was seen between locations, with it providing a high level of disease control at the Debert location compared to the Kemptown location. This could be due in part to there being a high probability of precipitation forecasted to occur within 48 hrs of fungicide application made on Kemptown plots. Due to the contact nature of folpet, and systemic nature of all other fungicide treatments, it could have been easily washed off the leaf surface. Rainfalls that occur soon after fungicide applications can dilute, redistribute or remove fungicide compounds from the leaf surface (Thacker & Young, 1999). When contact fungicides such as chlorothalonil and fluzinam were used in a factorial experiment to assess the effect of rainfall events at various times within an hour of fungicide application, it was found that rainfalls within an hour of application significantly reduced fungicide efficacy compared to when no rainfall occurred (Inguagiato & Miele, 2016). However, when the temporal

scale of these studies is broadened to rainfall events occurring 24 hours after fungicide application, rainfall appears to have less of an impact on fungicide residues. Cabras et al. (2001) conducted a study on grape leaves where folpet (Folpan® 80 WDG) residues were analysed followed controlled rainfall events. These authors found that rainfall of 15 or 30 mm administered 24 hours after fungicides were applied, did not reduce the folpet residues on the leaves. Rainfall of 45 mm 24 hours after fungicides were applied reduced the folpet residues by only 24%. Since folpet has limited diffusion into the epicuticular wax, the authors speculated that the rainfastness of folpet could be attributed to the surfactant's binding action to the leaf surface.

Across both locations and both assessment dates, the mixture of prothioconazole with benzovindiflupyr consistently reduced disease, but did not lead to increased harvestable berry yield. This treatment reduced percent leaves infected per stem, increased leaf number per stem and increased mean NDVI compared to other treatments. Mean floral bud numbers were not greater than all other treatments but were greater than many in this regard. Despite these results, prothioconazole with benzovindiflupyr did not have greater yields than other treatments. This treatment combination is however; used on a commercial basis, as it has been observed as being the best available option while conducting screening trials over multiple growing seasons (D. Percival, personal communication, Dalhousie University, NS, CA).

In other wild blueberry disease management studies, similar results have been observed where disease management treatments effectively reduce Botrytis blossom blight but no difference in berry yield compared to the untreated is documented. Abbey et al., (2022) found that burn pruning, calcium polysulphide and *Trichoderma harzianum* T-22

treatments applied in May, significantly reduced botrytis blight at full bloom when compared to the untreated control. However, when harvestable yield data were collected later in the growing season, there was no significant difference, and on occasion numerical difference between the disease management treatments and the untreated control. This may be an indication that confounding variables that naturally exist in wild blueberry fields such as blueberry species, plant density, phenotype, fertility and weed pressure can significantly influence harvestable berry yield (Kinsmen, 1993).

In the present study it can be noted that different fungicides with the same specific mode of action did not provide similar levels of control. The ability for fungicides to reduce disease can be strongly influenced by the sensitivity of the particular pathogen to the particular fungicide in question, rather than the fungicide's mode of action (Paul et al., 2008). For example, in this study all DMI's did not equally reduce disease. While all DMI fungicides reduce fungal growth by inhibiting the C-14 demethylase enzyme, the fungi's reaction to the fungicide can vary regardless of if they have the same mode of action (Breunig & Chilvers, 2022). This has been documented with other fungal species and fungicides using in-vitro tests to determine the sensitivity of select fungal pathogens. One example is when comparing the in-vitro sensitivity of prothioconazole and its major metabolite, desthio-prothioconazole. While both are considered DMI fungicides, specifically triazoles, desthio-prothioconazole provides greater fungal inhibition at a lower concentration (Breunig and Chilvers, 2022). Anatomically, the differences in triazole sensitivity can be characterized by the interaction of the compound's N-1 substituent group on the azole portion of the compound, to the CYP51 enzyme responsible for generating cell wall ergosterol (Strushkevich, Usanov, & Park, 2010).

Another potential reason to support the observation that different fungicides with the same mode of action did not provide similar levels of control is that the physiochemical properties of the compound may promote or prevent absorption into the plant. All of the fungicides used in this study, except folpet, are considered systemic but have varying degrees of absorption into the plant (Romeroc et al., 2020). How systemic a fungicide is can be dependent on physiochemical properties such the octanol-water coefficient (log K_{ow}) (Chollet et al., 2004). Compounds with a low logK_{ow} value have high lipophilicity meaning they can penetrate the cuticular wax (Augusto & Brenneman, 2012). Whereas a high logK_{ow} means that the compound may penetrate the waxy cuticular layer but may get bound to the wax layer and not get transported within the leaf (Edgington, 1981). Typically, logK_{ow} greater than 3.0 is considered non-mobile, whereas less than 3.0 is xylem mobile and able to readily pass through the cuticular layer (Satchivi, 2014). The logK_{ow} for prothioconazole is 2.0 making it xylem mobile, whereas for difenoconazole it is 4.4 considering it non-mobile. The logK_{ow} for other compounds in Protocol A in ascending order are 2.0 (prothioconazole), 3.02 (folpet), 3.13 (flupyroxad), 3.3 (fluopyram), 3.4 (mefentrifluconazole), 3.8 (pydiflumetofen), 4.3 (benzovindiflupyr) and 4.4 (difenoconazole). While the logK_{ow} can help describe the ability of a fungicide to penetrate the leaf's cuticular layer and be transported throughout the leaf, it is only one aspect of a complex relationship between fungicide, leaf and plant pathogens which can determine the efficacy of fungicides seen in the field.

2.5 Conclusion

As the regulations for pesticide use on wild blueberries changes, the management options for growers also needs to evolve. The results of the present study provide the wild

blueberry industry with a detailed outline of the effects of various fungicides on foliar diseases, leaf number, NDVI, fruit set, floral bud number and harvestable berry yield. The results indicate that foliar fungicides are an effective disease management option for wild blueberry growers. On average across assessment dates and locations, fungicide treatment prothioconazole with benzovindiflupyr reduced disease and maintained leaf number. Both fungicides are currently registered for use on wild blueberries. New fungicides, that are not registered for use on wild blueberries, such as mefentrifluconazole, pydiflumetofen and folpet provided some protection against diseases; however, further research investigating their use in combination with other fungicides is required. Additionally, as consumer demand for low pesticide residue products continues to grow there is a need to investigate cultural and biological disease management practices. There are increasingly more biological fungicides available on the market and determining their effectiveness on wild blueberry foliar diseases should be investigated.

CHAPTER 3

SENSITIVITY OF *SPHAERULINA VACCINII* AND *THEKOPSORA MINIMA* ISOLATES TO EXISTING AND NOVEL FUNGICIDES USED ON WILD BLUEBERRIES

3 Abstract

Foliar diseases of wild blueberries caused by *Thekopsora minima* and *Sphaerulina vaccinii* (previously *Septoria sp*.) are managed primarily using chemical control methods such as fungicides. Isolates of *S. vaccinii* were collected from 7 wild blueberry fields in 2021 and 2022, and *T. minima* urediniospores were collected from 4 wild blueberry fields in 2022 from across Nova Scotia. In this study, microplate light absorbance assay was used to assess *S. vaccinii* fungicide sensitivity, and urediniospore germination assay was used to assess *T. minima* fungicide sensitivity. Technical grade fungicides benzovindiflupyr, pydiflumetofen, prothioconazole, prothioconazole-desthio, and mefentrifluconazole were prepared at concentrations 0, 0.01, 0.1, 1.0, 10 and 100 $\mu g \cdot mL^{-1}$. The effective concentration which inhibits fungal growth by 50% (EC_{50}) was calculated using non-linear regression based on the relative growth. For *S. vaccinii*, the mean EC_{50} was 10.11, 4.62, 9.78, 0.82 and 6.76 $\mu g \cdot mL^{-1}$ for benzovindiflupyr, pydiflumetofen, prothioconazole, prothioconazole-desthio, and mefentrifluconazole, respectively. EC_{50} for prothioconazole-desthio was significantly lower than all other fungicides except pydiflumetofen. When comparing isolates collected in 2009 and 2022, it was observed that EC_{50} values did not significantly differ, except for prothioconazole which significantly increased ($P=0.014$). For *T. minima*, a significant interaction between fungicide and location was observed ($P<0.001$). All benzovindiflupyr x location treatments had a mean EC_{50} of 0.005 $\mu g \cdot mL^{-1}$, at least 98% lower than all other fungicide x location treatments. Mefentrifluconazole had the three greatest EC_{50} with 14.7, 17.3 and 23.9 $\mu g \cdot mL^{-1}$. Results showed that

demethylation inhibitor fungicides, prothioconazole, metabolite prothioconazole-desthio, and mefentrifluconazole, generally did not inhibit spore germination as effectively as the succinate dehydrogenase inhibitor fungicides, benzovindiflupyr and pydiflumetofen.

3.1 Introduction

Wild blueberry foliar diseases are especially problematic in the vegetative year of production and are primarily managed using fungicides. *Sphaerulina vaccinii* (previously *Septoria sp*.) and *Thekopsora minima* are the causal organisms of Sphaerulina leaf spot, Sphaerulina stem canker and blueberry leaf rust, respectively (Hildebrand, Renderos, & Delbridge, 2016; Ali et al., 2021). *S. vaccinii* is not known to infect any other economically important crops, while *T. minima* is present in highbush blueberry growing regions around the world and can now be found in western USA, parts of Europe, South America and Australia (Huarhua et al., 2020; Shands et al., 2018; Simpson et al., 2017; Wichura, Brand, & Böhm, 2020).

Chlorothalonil (Bravo® 500, Syngenta, Canada) was an effective fungicide option for foliar disease management in wild blueberries (Dawson, 2009). Chlorothalonil was used for a number of years independently but was found to provide greater disease control when tank mixed with prothioconazole. When prothioconazole became registered in Canada, the wild blueberry industry began using it in screening trials in conjunction with chlorothalonil and observed excellent results (D. Percival, personal communication, Dalhousie University, NS, CA). A tank mix of chlorothalonil and prothioconazole was widely used across the wild blueberry industry for managing foliar diseases. The European Union banned the use of chlorothalonil in Europe, primarily due to human health hazards (Arena et al., 2018) with Canada following suit in the winter of 2022 (Pest Management Regulatory

Agency, 2022). As chlorothalonil usage has been phased out, a tank mix of prothioconazole (Proline®, Bayer Ag) and benzovindiflupyr (Aprovia®, Syngenta Canada) has become the most common fungicide option for wild blueberry growers to manage foliar diseases.

Proline® (prothioconazole; Bayer Ag) was registered on wild blueberries in 2011 and is classified as a demethylation inhibitor (DMI) which inhibits sterol demethylation during the synthesis of ergosterols in the cell membrane (Parker et al., 2011). Aprovia® (benzovindiflupyr; Syngenta Canada) was registered on wild blueberry foliar diseases in 2016 and is classified as a succinate dehydrogenase inhibitor (SDHI) which is absorbed by growing fungal hyphae. It acts to inhibit fungal growth by blocking the activation site of succinate dehydrogenase, effectively stopping the electron transport chain process within the mitochondria of the fungal cell (Kuznetsov et al., 2018). The combination of prothioconazole and benzovindiflupyr has been documented to reduce foliar diseases by 48% compared to the untreated and have 17% greater leaf number than all other tested fungicide treatments up to sixty days after application (refer to Chapter 2).

3.1.1 Fungicide Resistance

As fungicides are introduced, they create a selection pressure in an agroecosystem, which requires fungicide resistance monitoring to ensure growers are still able to effectively manage diseases and receive sufficient return on investment when fungicides are used (Birr et al., 2021). A selection pressure refers to a biotic or abiotic variable that influences the ability for a population to reproduce, which can change the genetics of the population over time. Determining a pathogen's sensitivity to a particular fungicide *in vitro* also provides insight into the potential effectiveness of the fungicide at a field scale. If a particular pathogen appears to be sensitive to a fungicide *in vitro*, but does not have the

same result in field trials, it could indicate that the chemical is not being absorbed or translocated effectively within the plant. To track the efficacy of fungicides over time, an understanding of the baseline sensitivity is required and monitoring after the fungicide has been registered.

The development of fungicide resistant populations of plant pathogens has occurred on many crops around the world, including *Botrytis cinerea* on wild blueberries (Abbey et al., 2020; Müller, Stammler, & May De Mio, 2021). Fungicide resistance can develop in a population through two general pathways, qualitative and quantitative resistance. Qualitative resistance occurs when a specific mutation appears in a gene encoding a fungicide target protein that is required by the fungicide to inhibit the pathogen, resulting in a loss of fungicide efficacy (Deising, Reimann, & Pascholati, 2008). The individual from that population with the mutation will be able to survive and successfully reproduce in the presence of a fungicide, potentially outcompeting the wildtypes and leading to the entire population becoming resistant over multiple generations (Brent & Hollomon, 2007). Mutations occur spontaneously and naturally in all living things and is the basis for differences seen within a population and between populations. When this occurs, even a high concentration of fungicide will not inhibit the pathogen due to the change in proteins (Deising et al., 2008). Quantitative resistance can develop through mechanisms of the utilizing alternate metabolic pathways or producing enzymes to degrade the fungicide (Lesemann et al., 2006).

With the transition of fungicides from modes of action that inhibit fungal growth through multiple pathways to site-specific modes of action, there is increased risk of developing resistance (Brent & Hollomon, 2007). Factors that can influence fungicide

resistance include the quantity of fungicide used, number of applications per growing season and species biology (Brent & Hollomon, 2007). Prothioconazole and benzovindiflupyr are only applied once per growing season for managing foliar diseases on wild blueberries, sometimes twice if conditions are conducive for infection. While these are not optimal conditions for developing resistance, prothioconazole is also applied at other times in the growing season for managing *Monilinia vaccinii-corymbosi* which could act as a selection pressure for *S. vaccinii* and *T. minima* during other parts of their growth and reproductive cycles (Percival et al., 2017).

Pathogen sensitivity to fungicides can be assessed using a variety of techniques, including spore germination tests, mycelium colony growth diameter, light absorbance in a microtiter plate or molecular assay for simultaneous detection (Conti et al., 2019). Spore germination testing to assess fungicide sensitivity is completed by amending artificial growth media with fungicides at various concentrations and adding spores to the media (Chaulagain et al., 2019). After incubation, 100 spores are assessed for germination and the percent germination is used to determine the sensitivity. Mycelium colony growth is done by inoculating the center of a petri dish containing artificial growth media amended with fungicide. After incubation the colony diameter is measured and compared to that of the control (Gang et al., 2015). Microplate light absorbance is another technique that can assess fungicide sensitivity, where fungicide amended media are placed in the wells and inoculated. The light absorbance is measured using a microplate reader on the date of inoculation, and then again after incubation (McNab, Rether, & Hsiang, 2023). The change in absorbance can be used to quantify the fungicide sensitivity of the pathogen. The sensitivity of a pathogen to a select fungicide is typically assessed by determining the EC_{50},

which is the concentration of fungicide that inhibits growth by 50 percent (Koller & Scheinpflug, 1987).

Some fungicides have been observed to show greater inhibition of spore germination than mycelial growth, or visa versa. During a pathogen's growth, spore germination is often highly sensitive to inhibition by fungicides (Slawecki, Ryan, & Young, 2002), which is beneficial because this can prevent fungal pathogens from entering the plant tissue and causing disease symptoms. However, not all fungicides are effective at inhibiting spore germination, with some DMI fungicides not inhibiting spore germination (Slawecki et al., 2002) but being more successful at inhibiting mycelial growth (Golembiewski et al., 1995). On the other hand, some SDHI fungicides exhibit better inhibition of spore germination than mycelial growth (Xu et al., 2019). With this in mind, the mechanism by which fungicides prove to be more inhibitive based on the assay method used, needs to be noted when comparing EC_{50}.

3.1.2 Objectives

The purpose of this study was to determine the fungicide sensitivity of *S. vaccinii* and *T. minima* isolates to prothioconazole, prothioconazole-desthio, benzovindiflupyr, mefentrifluconazole and pydiflumetofen using a collection of isolates from commercial wild blueberry fields across Nova Scotia.

3.2 Materials and Methods

3.2.1 Fungal Isolation

S. vaccinii was isolated from diseased leaves and stem cankers collected from 7 commercial wild blueberry fields across Nova Scotia. Leaf lesions and stem cankers were used because they correspond to the same causal organism, and both play a role in the

organisms life cycle. Fields were located in Debert, Kemptown, East River Saint Marys, Mount Thom, Glasgow Mountain, Londonderry and North River. Sampling was not done systematically but rather by finding infected plants throughout a field. When multiple isolates were collected from one field, they were not isolated from the same plant. Small portions of the infected leaf tissue and stem canker were separated from the healthy part of the plant using a scalpel. Infected cuttings were surface sterilized by immersing in 2% sodium hypochlorite (NaOCl) for 1-2 minutes, rinsing in sterile water twice and drying on sterile filter paper (Barrau, de los Santos, & Romero, 2001; Castillo et al., 2013; Kharbanda & Bernier, 1980). Once dry, leaf cuttings were placed on potato dextrose agar (PDA) amended with streptomycin at a concentration 50 μg mL^{-1} to prevent bacterial contamination and incubated in the dark at 22°C for 7 days. Fungal colonies that resembled *S. vaccinii* colonies were viewed under a compound light microscope (Leitz Diaplan, Germany) to determine the presence and shape of conidia compared to that described by Ali et al. (2021). Colonies that appeared to be *S. vaccinii* were subcultured to fresh plates of PDA (Figure 3.1). Three additional *S. vaccinii* isolates that were collected in 2009, prior to the use of any current fungicides, were removed from cold storage and used in this study. More isolates from 2009 would have been used; however, issues with contamination of stored isolates occurred.

To evaluate *T. minima* urediniospore germination in the presence of fungicides, soil plugs with plants exhibiting signs or symptoms of *T. minima* infection were removed from 4 wild blueberry fields across Nova Scotia in September of 2022. Four plugs per location were removed. Plants were moved into growth chambers (Conviron PGR15, Winnipeg, Manitoba) where they were incubated for 14-days at 18°C and 90% RH with 16 hr

photoperiod. Uredia development on the abaxial leaf surface was assessed daily. Once enough uredia were present on the underside of leaves, infected leaves were removed from the growth chamber and placed in an airtight plastic container with damp paper towel to maintain humidity until required for germination testing (Figure 3.2). Leaves were not kept in plastic containers for longer than 24 hours.

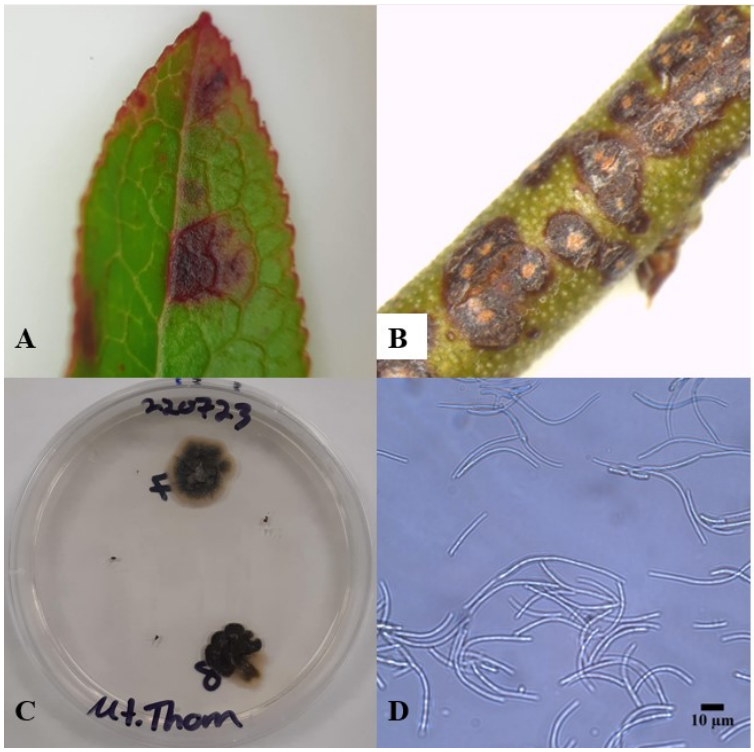

Figure 3.1. (A) Lesions on wild blueberry leaves caused by *S. vaccinii*, (B) stem cankers on wild blueberry stems caused by *S. vaccinii*, (C) leaf cuttings of lesions placed on PDA and incubated for 14 days with fungal colonies forming, (D) conidia used to prepare conidia suspension and inoculate microplate wells. Infected leaves and stem canker collected from commercial wild blueberry fields in Nova Scotia, CA.

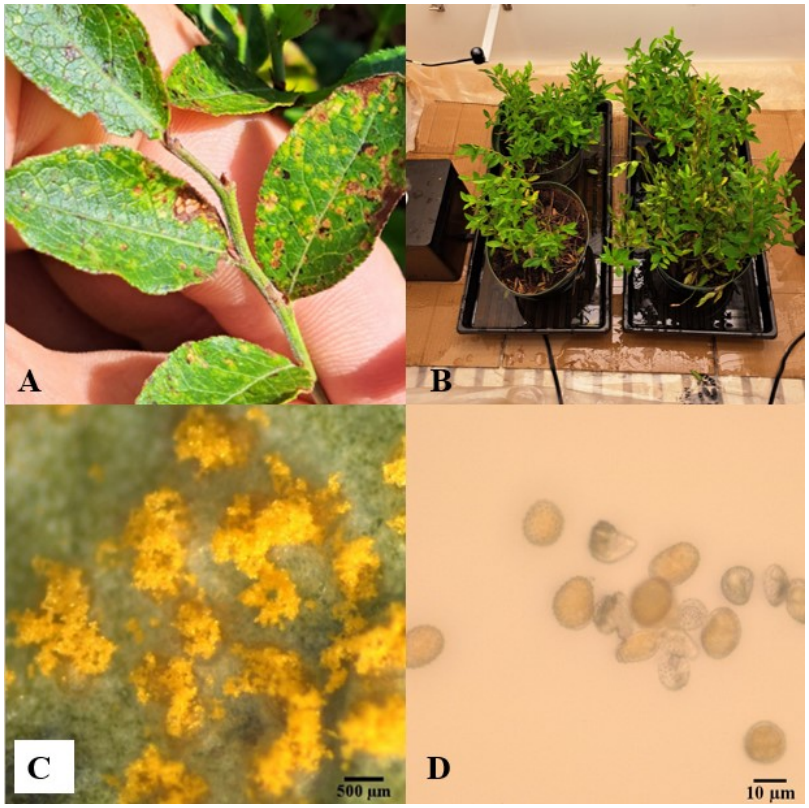

Figure 3.2. (A) Immature lesions on wild blueberry leaves caused by *T. minima*, (B) soil plugs with blueberry plants removed from the field and grown in the growth chamber at 18°C and 90% RH, (C) masses of urediniospores on the abaxial leaf surface, (D) urediniospores viewed on a microscope slide.

3.2.2 Fungicide Sensitivity

Sensitivity of *S. vaccinii* to prothioconazole, prothioconazole-desthio, mefentrifluconazole, benzovindiflupyr and pydiflumetofen fungicides was assessed in black, clear bottom, 96-well microplates (200 µL wells) (Xavier et al., 2021). Technical-grade fungicides (>97% purity) were dissolved in acetone to form stock solutions. The final acetone concentration in the amended media was 1 $\mu L \cdot mL^{-1}$ (0.1% v/v). The sensitivity of each isolate was determined by growing each *S. vaccinii* isolate in liquid yeast sucrose media (LYSM) (10g sucrose, 10 g yeast extract in 1 L deionized water) amended with fungicide to provide resulting concentrations of 0, 0.01, 0.1, 1, 10 and 100 $\mu g \cdot mL^{-1}$. Streptomycin was also added to LYSM at a uniform rate of 50 $\mu g\ mL^{-1}$ to prevent bacterial

contamination. One-hundred fifty µL of LYSM amended with fungicide was amended with fungicide was added to each well, with 50 µL of *S. vaccinii* conidia suspensions (10^6 spores mL^{-1}) added to each well. The addition of conidia suspension was accounted for in the fungicide concentrations. Each isolate-fungicide concentration combination was replicated 4 times and plates were set up as a complete randomized design. Directly following the addition of LYSM and conidia suspension to the microplate wells, lids were placed on the plates and sealed with parafilm (Parafilm® M PM-996 All Purpose Laboratory Film). The absorbance reading was taken immediately after placing the lid on the microplate (day 0) at 450 nm using a BioTek Synergy H1 Plate Reader (BioTek® Instruments, Inc. Winooski, Vermont). Microplates were incubated at 22℃ for 10 days with constant agitation at 100 rpm (Cole-Parmer Benchtop Orbital Shaker). Plates were visually checked for contamination before absorbance was measured after 10 days of incubation. Wells that were contaminated were not included in the relative growth calculations or statistical analysis. The change in absorbance was calculated by subtracting the day 0 reading from the day 10 reading. Relative growth was calculated by the equation [1 – (the mean change in absorbance on amended media divided by the mean change in absorbance on unamended media)] and transformed into a percent.

Sensitivity of *T. minima* to prothioconazole, prothioconazole-desthio, mefentrifluconazole, benzovindiflupyr and pydiflumetofen fungicides was determined using urediniospore germination tests, similar to that of Li et al. (2009). The same fungicide stock solution as the *S. vaccinii* testing was added to molten half strength PDA (Li et al., 2009). The final acetone concentration in the amended PDA was 1 $\mu L \cdot mL^{-1}$ (0.1% v/v). Half strength PDA was amended with fungicides to final concentrations of 0, 0.01, 0.1, 1,

10 and 100 μg·mL^{-1}. Aliquots of 1.0 mL fungicide amended half strength PDA were pipetted onto microscope slides. Once the half strength PDA was solidified, urediniospores were dusted onto the media. To remove urediniospores from infected leaves, urediniospores were brushed directly from the underside of the leaves using a camel's hair brush. Once inoculated, slides were incubated in the dark at 22°C for 24 hours in airtight plastic containers with damp paper towel to increase humidity. Each concentration x location combination was replicated 4 times. Using a compound light microscope (Leitz Diaplan, Germany) and image analysis software (Leica LAS X v3.014, Germany) 100 spores were analysed for germination. Urediniospores were considered germinated if the germ tube was longer than or equal to the short diameter of the spore (Pfister, Halik, & Bergdahl, 2004) . Relative growth was calculated by the equation [1 – (the mean percent spore germination on amended media divided by the mean percent spore germination on unamended media)] and transformed into a percent.

3.2.3 Statistical Analysis

To calculate the EC_{50} (μg mL^{-1}) for each *S. vaccinii* and *T. minima* isolate, a linear regression was completed for relative growth against log of the fungicide concentration using SigmaPlot (version 12.0, software Inc., San Jose, California, US). Using the EC_{50} values, the normality of the error terms and constant variance were verified using the residuals in a normal probability plot (NPP) of residuals using the Anderson-Darling test ($\alpha = 0.05$), and residuals versus fitted values plot. If the error terms were not normal or did not have constant variance, the data was transformed. Minitab (version 19.1.1) was used to check normality and constant variance and to make any transformations.

Once normalized, EC_{50} values were analysed using PROC GLIMMIX procedure of SAS (version 9.4, SAS institute, Inc., Cary, NC) to assess the main and interactive effects. The main effects were location and fungicide. If there was a significant interaction effect a multiple means comparison was done using Tukey honestly significant difference (HSD) at $\alpha = 0.05$. If there was no significant interaction effect, a multiple means comparison with Tukey HSD at $\alpha = 0.05$ was done for significant main effects. Each assessment date and protocol were analysed separately, and location was analysed separately when a treatment by location interaction was found.

A two-sample t-test was used to compare the difference in EC_{50} concentrations between the 2021/2022 isolates and the 2009 isolates for each fungicide using PROC TTEST procedure of SAS (version 9.4, SAS institute, Inc., Cary, NC) at $\alpha=0.05$.

3.3 Results and Discussion

3.3.1 *Sphaerulina vaccinii* Sensitivity

EC_{50} concentrations were transformed using the $Y^{(1/3)}$ transformation to normalize error terms (Anderson-Darling $p = 0.19$). Non-transformed EC_{50} concentrations are displayed in Table 3.1. Isolates from Debert had to be removed from the GLM assessing the main and interactive effects because the treatment Debert x benzovindiflupyr did not have any EC_{50} values due to microplate well contamination. EC_{50} was significantly impacted by the fungicide main effect ($P < 0.001$) but not location, and there was no significant interactive effect ($P = 0.37$) (Table 3.2). The mean fungicide EC_{50} values were significantly different, with prothioconazole-desthio having significantly lower mean EC_{50} values than benzovindiflupyr, prothioconazole and mefentrifluconazole. Fungicides that

have not been registered or registered for the 2022 season did not have significantly lower EC_{50} values than fungicides that have been registered for over 10 years (Table 3.3).

Prothioconazole-desthio had a mean EC_{50} that was 92% lower than that of prothioconazole (Table 3.3). Previously, it has been found that prothioconazole-desthio is more efficacious than prothioconazole while conducting sensitivity testing for *Fusarium graminearum* (Breunig & Chilvers, 2022). This difference has been attributed to the activity of prothioconazole-desthio binding noncompetitively to the CYP51 enzyme and exhibiting typical enzyme inhibition, whereas prothioconazole does not inhibit CYP51 activity and binds competitively to the CYP51 enzyme (Breunig & Chilvers, 2022). The greater efficacy in-vitro of prothioconazole-desthio compared to prothioconazole is beneficial to managing diseases in the field because prothioconazole degrades into its metabolite over time. When wheat flag leaves were treated with prothioconazole, the ratio of prothioconazole to prothioconazole-desthio was 1:1.71-1.97 when assessed 8 days after application (Lehoczki-Krsjak et al., 2013).

Newly registered fungicide pydiflumetofen and not yet registered fungicide mefentrifluconazole did not exhibit significantly lower EC_{50} compared to fungicides that have been used for over 10 years (Table 3.3). The small sample size of 2009 isolates needs to be considered when comparing 2009 and 2022 isolates. Due to contamination and disruptions in storage temperature, only three 2009 isolates were able to be revived from storage. Pydiflumetofen was first registered for use on wild blueberries in 2021 under the trade name Miravis® Neo. Prothioconazole has been registered on wild blueberries for 11 years and benzovindiflupyr has been registered for 4 years. It can be hypothesized that 11 years is not a significant amount of time for resistance to develop given the single

application (sometimes two) per year for managing foliar diseases. Other sensitivity testing studies have found that it takes closer to 20 years of widespread use to see noticeable changes in the population's EC_{50}; however, this would depend on the number of applications and species in question (Birr et al., 2021). It can also be hypothesized that although the active ingredients are different, they still have the same mode of action (i.e. prothioconazole and mefentrifluconazole) which biochemically inhibit fungal growth in the same manner. EC_{50} values in the present study appear to be more strongly influenced by the specific chemical, rather than how long it has been in use for. This is also observed when 2009 and 2022 isolates were compared (Table 3.4), keeping in mind that only three 2009 isolates were used due to contamination and disruptions in storage.

Table 3.1 The mean EC_{50} values of *Sphaerulina vaccinii* isolates collected from commercial wild blueberry fields across Nova Scotia for registered and novel fungicides used for foliar disease management (n=4).

		EC_{50} (µg·mL^{-1})**				
Isolate*	**Year**	**Benzo**	**Pydi**	**Proth**	**Desthio**	**Mefen**
Debert 1	2022	-	0.23	6.73	0.12	1.72
Debert 2	2022	-	0.60	4.73	0.63	4.28
Debert 3	2022	-	0.002	12.34	0.63	1.72
Kemptown 1	2022	11.05	0.73	3.68	0.44	4.28
Kemptown 2	2022	9.52	1.02	10.46	0.97	4.23
Kemptown 3	2022	3.03	1.57	10.99	1.26	0.13
Kemptown 4	2022	-	2.70	14.02	0.06	0.07
East River Saint Marys 1	2022	0.98	1.45	42.28	0.37	0.06
East River Saint Marys 2	2022	1.08	0.23	1.44	0.07	1.28
East River Saint Marys 3	2022	1.67	2.65	6.15	0.51	1.11
East River Saint Marys 4	2022	5.07	4.56	26.71	0.2	5.82
Mount Thom 1	2022	27.59	1.96	9.47	1.72	4.91
Mount Thom 3	2022	2.29	0.66	-	0.14	11.85
Mount Thom 4	2022	0.44	0.63	4.64	0.63	20.43
Glasgow Mountain 1	2022	3.70	0.35	5.00	1.08	1.23
Glasgow Mountain 3	2022	22.54	0.28	7.58	0.91	1.01
Glasgow Mountain 4	2022	3.10	1.25	9.99	0.1	55.68
Londonderry 1	2022	23.25	0.25	10.24	1.94	0.64
Londonderry 2	2022	22.67	0.60	23.11	4.01	-
Londonderry 4	2022	7.34	3.03	-	1.94	20.09
North River 1	2022	8.09	0.09	5.72	0.31	1.82
North River 2	2022	24.87	-	2.93	0.94	6.65
East River Saint Marys 7	2009	18.82	0.96	0.81	0.09	0.27
East River Saint Marys 21	2009	10.35	3.29	1.69	0.69	7.43
East River Saint Marys 43	2009	4.95	4.95	4.24	0.77	5.47

*Isolate = collection location, isolate number.

**Benzo = Benzovindiflupyr, Pydi = pydiflumetofen, Proth = prothioconazole, Desthio = prothioconazole-desthio, Mefen = mefentrifluconazole.

73

Table 3.2 The main and interactive effects of fungicide and location on the EC_{50} ($\mu g \cdot mL^{-1}$) for sensitivity tests on *Sphaerulina vaccinii* (N=118) and *Thekopsora minima* (N=59) using a general linear model (α=0.05).

	Fungal Species	
ANOVA source of variation	***Sphaerulina vaccinii***	***Thekopsora minima***
Two-way ANOVA main effects		
Active Ingredient	<0.001	<0.001
Location	0.43	0.73
Two-Way ANOVA Interactions		
Active Ingredient x Location	0.26	<0.001

Table 3.3 Mean *Sphaerulina vaccinii* EC_{50} ($\mu g \cdot mL^{-1}$) calculated using isolates collected from 8 wild blueberry fields across Nova Scotia. The results of ANOVA ($p<0.001$) show there is a significant difference between the sensitivity of *Sphaerulina vaccinii* to select fungicides. The number of isolates varies because some microplate wells became contaminated and some EC_{50} were considered outliers, therefore they were not included in the analysis.

Fungicide	**Number of Isolates**	**EC_{50} ($\mu g \cdot mL^{-1}$)**	
		Range	**Mean[z] (±SE)**
Benzovindiflupyr	21	(0.44-27.59)	10.11 (±2.00) a
Pydiflumetofen	25	(0.002-4.95)	1.42 (±0.29) bc
Prothioconazole	23	(1.44-42.28)	9.78 (±1.99) a
Prothioconazole-desthio	25	(0.07-4.01)	0.82 (±0.17) c
Mefentrifluconazole	24	(0.06-55.68)	6.76 (±2.42) ab

[z] Mean EC_{50} values calculated using nonlinear regression for each active ingredient. Mean EC_{50} followed by different letters are significantly different ($p<0.05$) according to the Tukey HSD pairwise comparison. Tukey HSD pairwise comparison was done using transformed values, however the values shown in the table are from non-transformed data.

Table 3.4 Mean *Sphaerulina vaccinii* EC_{50} ($\mu g \cdot mL^{-1}$) for 2009 and 2022 isolates compared using a two-sample t-test (α=0.05).

	Mean[z] EC_{50} ($\mu g \cdot mL^{-1}$) (±SE)			T-Test Results
Fungicide	**2009**	**2022**	**Transformation**	**(p-Value[x])**
Benzovindiflupyr	11.37 (±4.04)	9.90 (±2.21)	$Y^{1/2}$	NS
Pydiflumetofen[y]	3.07 (±1.16)	1.18 (±1.88)	$Y^{1/2}$	NS
Prothioconazole[y]	2.24 (±1.03)	10.91 (±2.29)	$Y^{1/2}$	0.014
Prothioconazole-desthio	0.52 (±0.22)	0.86 (±0.22)	$Y^{1/2}$	NS
Mefentrifluconazole[y]	4.39 (±2.14)	7.10 (±3.18)	$Y^{1/3}$	NS

[z] Mean EC_{50} values calculated using nonlinear regression for each active ingredient and year collected.
[x] The results of the two-sample t-test were not significant (NS) if p>0.05.
[y] Unequal variance for 2009 and 2022 isolates so t-test is approximate.

There were no significant differences between the locations and the sensitivity of isolates from those locations to the various fungicides or modes of action (P = 0.43) (Table 3.2). This is not overly surprising as most of the fields where isolates were collected from have received similar fungicide treatments in the past. In addition, there was high variability of EC_{50} values within a location, this was consistently seen at all locations (Table 3.1). This possibly indicates high natural variation for this species to fungicides. In other studies, examining fungicide sensitivity the variation between and within locations is comparable in magnitude. For example, Breunig & Chilvers (2021) found high variation in EC_{50} values when isolates of *Fusarium graminearum* from across Michigan were tested for baseline sensitivity to pydiflumetofen. It was found that the mean EC_{50} was 0.6 $\mu g \cdot mL^{-1}$, with values ranging from 0.008 to 0.263 $\mu g \cdot mL^{-1}$.

An alternative reason for the high variation seen within a location could be that the collected isolates may in fact not have the same exposure history and the more sensitive isolates are wildtypes that have recently spread into the commercial fields. Since *Vaccinium angustifolium* and *Vaccinium myrtilloides* exist in the forests surrounding the wild blueberry fields, there is a possibility that inoculum can spread between the commercial

fields and unmanaged blueberry plants in the neighbouring forests. The wild blueberry plants found in neighbouring forests often have some disease symptoms resembling those caused by *S. vaccinii*.

Another notable result from the present study was that the calculated EC_{50} values were much greater than many other studies that examined fungicide sensitivity. For example, Birr et al., (2021) studied temporal changes in *Zymoseptoria tritici* sensitivity to DMI and SDHI fungicides in Europe. DMI fungicides were first registered for use on *Z. tritici* in the 1980s, with the first sensitivity testing conducted in 1999 and every ten years there after. The initial sensitivity testing done in 1999 showed that the mean EC_{50} was below 1.0 $\mu g \cdot mL^{-1}$ for all tested DMI and below 0.1 $\mu g \cdot mL^{-1}$ for SDHI fungicides which were not registered until the mid-2000's. In their study it was not until 20 years of widespread use did the mean EC_{50} begin to exceed 1.0 $\mu g \cdot mL^{-1}$ for DMI and 0.1 $\mu g \cdot mL^{-1}$ for SHDI fungicides (Birr et al., 2021). In comparison, the present study found that *S. vaccinii* sensitivity to DMI fungicides had EC_{50} concentrations of 9.78, 0.82 and 6.76 $\mu g \cdot mL^{-1}$ for prothioconazole, prothioconazole-desthio and mefentrifluconazole, respectively (Figure 3.3). The SDHI fungicides had EC_{50} concentrations of 10.11 and 1.42 $\mu g \cdot mL^{-1}$ for benzovindiflupyr and pydiflumetofen, respectively (Figure 3.3).

3.3.2 *Thekopsora minima* Sensitivity

EC_{50} values were transformed using the $Y^{-1/2}$ transformation to normalize the error terms. EC_{50} was significantly impacted by the fungicide main effect ($P < 0.001$), and there was significant fungicide x location interactive effect ($P < 0.001$) (Table 3.2). An ANOVA of the two-way interaction was completed, followed by a multiple mean comparison using Tukey HSD. The results show that all benzovindiflupyr x location treatments had at least

98.4% lower EC_{50} concentrations when compared to all other fungicide x location treatments (Table 3.5). Differences in germination rates between fungicides can be seen in Figure 3.3 and 3.4. When spore germination was assessed with benzovindiflupyr at 10 $\mu g \cdot mL^{-1}$ the urediniospores did not germinate and appeared to have lost their structure (Figure 3.3C).

Benzovindiflupyr was highly effective at inhibiting urediniospore germination, having an overall mean EC_{50} concentration of 0.005 $\mu g \cdot mL^{-1}$. In the field, benzovindiflupyr has been noted as being an effective SDHI fungicide for managing other rust diseases around the world (Carmona et al., 2020). Fungicide sensitivity of *Phakopsora pachyrhizi*, the causal organism of Asian soybean rust, has been assessed using urediniospore germination assay test for a variety of common fungicides (Juliatti et al., 2017). It was found that *P. pachyrhizi* spore germination rate was inhibited by 11% at benzovindiflupyr concentration of 1.0 $\mu g \cdot mL^{-1}$ and 77% at 50 $\mu g\ mL^{-1}$. In comparison, the present study found the *T. minima* spore germination rate was inhibited by 97% at 1.0 $\mu g \cdot mL^{-1}$ and 98% at 10 $\mu g \cdot mL^{-1}$. Similar results were found when Müller et al. (2021) used detached leaves to measure benzovindiflupyr EC_{50} concentrations. Using 21 isolates, they determined the average EC_{50} was 8.6 $\mu g \cdot mL^{-1}$, compared to the present study where it was 0.005 $\mu g \cdot mL^{-1}$.

Table 3.5 The results for an ANOVA (n=4) for mean EC_{50} (µg mL^{-1}) of *Thekopsora minima* populations collected from 4 wild blueberry fields in Nova Scotia. The general linear model results indicated there is a two-way interaction between the main effects, fungicide and location (P<0.001).

		EC_{50} (µg·mL^{-1})	
Fungicide	**Location**	**Range**	**Mean[z] (±SE)**
Benzovindiflupyr	Debert	($4.60x10^{-5}$ - 0.035)	0.011 (±0.006) a
	Londonderry	(0.0004 - 0.002)	0.001 (<0.001) a
	Mt. Thom	(0.0001 - 0.025)	0.007 (±0.004) a
	Kemptown	(0.0001 - 0.004)	0.002 (±0.001) a
Pydiflumetofen	Debert	(1.89 - 22.0)	8.66 (±4.33) de
	Londonderry	(0.67 - 1.12)	0.840 (±0.42) abc
	Mt. Thom	(0.29 - 1.46)	0.650 (±0.33) ab
	Kemptown	(1.16 - 2.55)	1.75 (±0.88) bcd
Prothioconazole	Debert	(1.32 – 2.85)	3.18 (±1.3) bcd
	Londonderry	(4.47 – 16.6)	9.75 (±4.9) de
	Mt. Thom	-	-
	Kemptown	(1.78 – 12.7)	5.77 (±2.9) cde
Prothioconazole-desthio	Debert	(4.01 – 21.9)	9.84 (±4.9) de
	Londonderry	(2.58 – 4.91)	3.56 (±1.8) cde
	Mt. Thom	(0.81 – 8.69)	3.71 (±1.9) bcde
	Kemptown	(3.68 – 5.92)	4.57 (±2.3) cde
Mefentrifluconazole	Debert	(2.82 - 46.0)	14.7 (±7.2) de
	Londonderry	(7.85 - 12.3)	9.4 (±8.6) de
	Mt. Thom	($4.04x10^{-33}$ - 63.3)	17.3 (±8.6) de
	Kemptown	(14.3 - 32.9)	23.9 (±12.0) e

[z] Mean EC_{50} values calculated using nonlinear regression for each active ingredient. Mean EC_{50} followed by different letters are significantly different (p<0.05) according to the Tukey HSD pairwise comparison. Tukey HSD pairwise comparison was done using transformed values, however the values shown in the table are from non-transformed data.

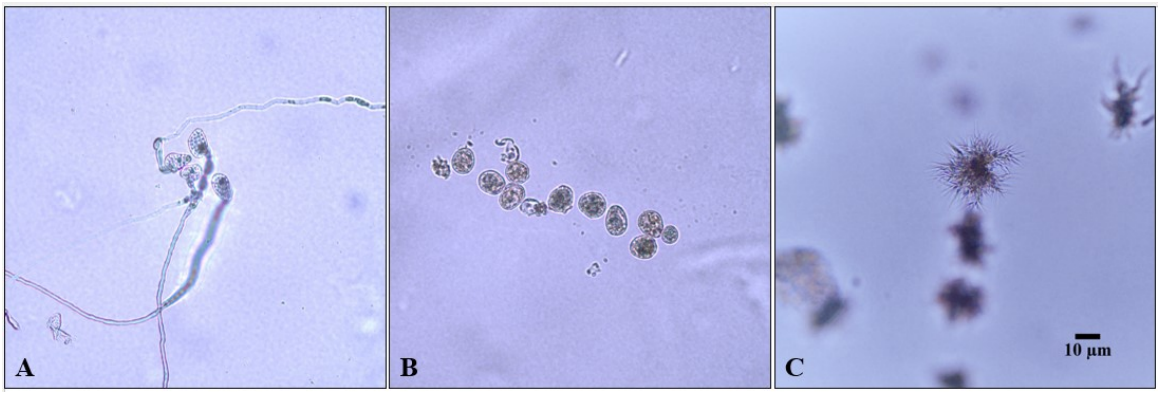

Figure 3.3 *T. minima* urediniospore germination 24 hours after incubation on media amended with benzovindiflupyr at 0 (A), 0.1 (B) and 10 (C) µg·mL^{-1}.

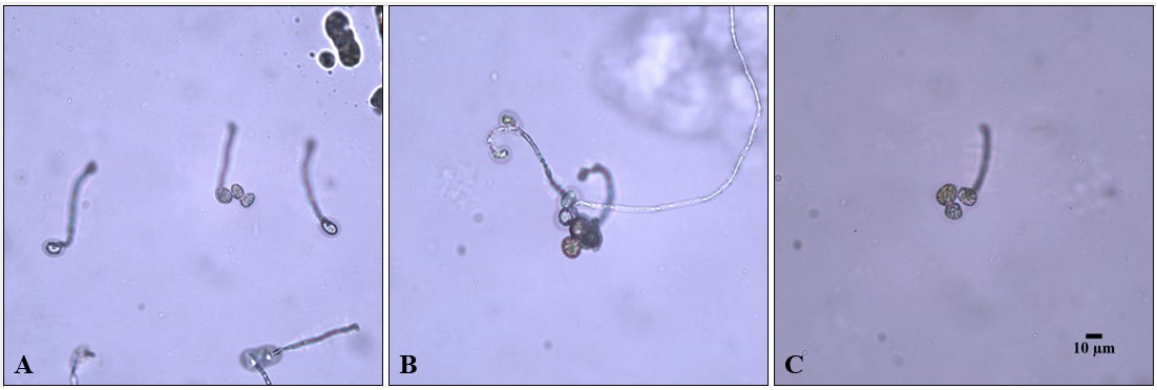

Figure 3.4 *T. minima* urediniospore germination 24 hours after incubation on media amended with prothioconazole-desthio at 0 (A), 0.1 (B) and 10 (C) µg·mL^{-1}.

The difference in EC_{50} values could be a result of varying degrees of previous exposures to benzovindiflupyr or differences in the fungal species response to fungicides. For *P. pachyrhizi*, isolates were collected in 2017, from regions of Brazil where this fungicide was registered in 2013. In the Brazilian soybean cropping system, fungicides are typically applied multiple times within a growing season (Juliatti & Zambolim, 2021). There were reports of reduced efficacy of SDHI fungicides in those regions in 2016, just three years after its registration. However, when isolates were assessed for the SdhC I86F mutation, one of gene mutations responsible for resistance to SDHI fungicides, the majority of isolates were found to be wildtypes and did not possess the I86F mutation and therefore were not the result of point mutations (Müller et al., 2021). Differences in the fungal species

could be the cause of the differences seen between the present study and sensitivity of *P. pachyrhizi*. Spores from *P. pachyrhizi* and *T. minima* would utilize the same respiratory pathways to generate ATP and germinate, however as it has been shown in many other studies, sensitivity can depend on the species in question (Iacomi-Vasilescu et al., 2004; Slawecki et al., 2002). An example of this was documented by Slawecki et al. (2002) where *Botrytis cinerea* and *Puccinia recondita* were evaluated for spore germination in the presence of fungicides under the same laboratory conditions. The resulting EC_{50} values varied between the two species despite the parallel lab conditions.

Another important trend in the present study was that DMI fungicides generally did not inhibit spore germination as effectively as the SDHI fungicides. For the SDHI fungicides, the EC_{50} was 0.005 and 2.9 $\mu g \cdot mL^{-1}$ for benzovindiflupyr and pydiflumentofen, respectively. In comparison to the DMI fungicides, the EC_{50} was 6.2, 5.4 and 16.4 $\mu g \cdot mL^{-1}$ for prothioconazole, prothioconazole-desthio and mefentrifluconazole, respectively. Early studies of the effects of DMI fungicides on sterol biosynthesis revealed that they have little effect on spore germination (Siegel, 1981; Slawecki et al., 2002). When urediniospore germination has been tested in the presence of fungicides on artificial media, germination rates do not appear to decline significantly until concentrations reached in the magnitude of 70 $\mu g \cdot mL^{-1}$ (Kang et al., 2019). Despite multiple reports of minimal effect on spore germination, an alternative study using DMI fungicides tebuconazole and triadimenol found that *Puccinia graminis* f. sp. *tritici* urediniospore germination declined at a concentration of 1 $\mu g \cdot mL^{-1}$. Upon investigation into the sterol synthesis during urediniospore germination, it was found that *P. graminis* f. sp. *tritici* urediniospores used their own sterol reserves to develop germ tubes and did not require supplemented sources

of sterols. The DMI fungicides were documented as completely blocking sterol C-14 demethylation, however since the spores had sterol reserves, it is thought that spores were not required to synthesize more during spore germination (Pontzen & Scheinpflug, 1989). The sterol composition within fungal organisms has been observed to be different across phyla, however little research has been done investigating differences in sterol composition within phyla (Weete, Abril, & Blackwell, 2010). With that in mind, determining if the differences in urediniospore germination rates in the present study, compared to other studies, are a result of differences in ergosterol composition within the urediniospores, cannot be concluded.

3.4 Conclusion

The present study revealed that fungicide sensitivity for *S. vaccinii* and *T. minima* varied depending on the fungicide and was less impacted by the location where the isolates were collected. *S. vaccinii* isolates were highly sensitive to prothioconazole-desthio compared to other fungicides and especially compared to its parent compound prothioconazole. Propiconazole EC_{50} values for *S. vaccinii* observed by Burkaloti and Percival (2023) were very similar to prothioconazole EC_{50} values calculated for 2009 isolates in the present study. When three 2009 isolates from East River St. Marys, NS were compared to isolates collected in 2021 and 2022, no significant differences were seen between EC_{50} for all fungicides except prothioconazole. For the SDHI fungicides benzovindiflupyr and pydiflumetofen, the EC_{50} actually decreased from 2009 to 2021/2022 showing that resistance to these fungicides has likely not occurred over this timescale. The results from the *S. vaccinii* sensitivity test also show that the microplate light absorbance method is an effective method for fungicide testing with this organism. Regarding *T.*

minima, isolates were most sensitive to benzovindiflupyr compared to all other fungicides. The DMI fungicides prothioconazole, prothioconazole-desthio and mefentrifluconazole did not appear to greatly inhibit spore germination. *T. minima* sensitivity results should be considered in the context that urediniospore germination is only one small step in the fungal life cycle. Due to the obligate nature of this organism conducting tests using mycelial growth radius or microplate light absorbance were not possible. The sensitivity tests completed here serve as a starting point for further fungicide sensitivity monitoring in wild blueberry fields and can be used in assessing temporal changes.

The observed sensitivity testing results provide insight into how these fungicides inhibit certain stages of the pathogens growth and can help guide management decisions in the field. One notable result was the high efficacy of benzovindiflupyr at inhibiting urediniospore germination. With this in mind, targeting field applications of benzovindiflupyr with urediniospore dispersal and infection period could increase the efficacy of benzovindiflupyr in the field. To an extent, this is already being done since the application of fungicides prothioconazole with benzovindiflupyr for managing foliar diseases is made in late July, which corresponds to the timing of urediniospore dispersal in early August (Nguyen, 2019). As well, the decrease in prothioconazole sensitivity over time (and when compared to propiconazole sensitivity testing in 2009) shows that the sensitivity of *S. vaccinii* to prothioconazole and other DMI fungicides should be monitored in the field and *in vitro* in the future for reduced efficacy. Although prothioconazole is typically only used once per season for managing foliar diseases, it is used in crop year fields for management of Monilinia blight. If other modes of action are registered and are efficacious for Monilinia blight, it would be recommended to use those fungicides as

opposed to prothioconazole as to not increase the chance of resistance development for *S. vaccinii*.

CHAPTER 4

QUANTITATIVE DETERMINATION OF FOLIAR FUNGICIDE PERSISTENCE AND DISSIPATION IN WILD BLUEBERRY LEAVES USING GAS AND LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

4 Abstract

The persistence and dissipation rate of foliar fungicides used for managing wild blueberry foliar diseases was assessed. Extraction of fungicides from leave tissue was completed using a quick, easy, cheap, effective, rugged and safe (QuEChERS) followed by solid phase extraction (SPE). Fungicides pydiflumetofen and benzovindiflupyr were assessed using gas chromatography-tandem mass spectrometry (GC-MS) while, prothioconazole, prothioconazole-desthio and mefentrifluconazole were assessed using liquid chromatography-tandem mass spectrometry (LC-MS). Blueberry leaf samples were collected 2 hrs after application, and then again 7, 14 and 30 days after application (DAA). For pydiflumetofen, prothioconazole-desthio and mefentrifluconazole, it was observed that the concentration detected 7 DAA was greater than the initial residue detected 2 hours after application, however concentrations decreased beyond 7 days. After 30 days, residues for pydiflumetofen, benzovindiflupyr, prothioconazole-desthio and mefentrifluconazole were 33.8, 18.4, 33.3 and 50.7% of their initial detected mean concentration, respectively. Using the first-order reaction kinetics, the half-life for of pydiflumetofen, benzovindiflupyr, prothioconazole-desthio and mefentrifluconazole were calculated as 19.8, 12.6, 34.7, and 23.9 days, respectively. Prothioconazole was unable to be detected with the LC-MS methodology used.

4.1 Introduction

4.1.1 Chemical Control Methods

Over the last 20 years, wild blueberry foliar diseases have become highly problematic in the vegetative year of production. Foliar diseases such as blueberry leaf rust and Sphaerulina leave spot caused by *Thekopsora minima* and *Sphaerulina vaccinii*, respectively, can reduce photosynthesis, net carbon exchange rate, leaf respiration rate, leaf number, floral bud number and yield (Dawson, 2009; see Chapter 2). Alone these diseases can cause yield losses of up to 60% when left unmanaged in the vegetative year (Percival & Dawson, 2009). Currently, there are limited cultural or biological management strategies, so management of these diseases is highly dependent on fungicides.

In the vegetative year, fungicides to manage foliar pathogens are applied once during the growing season, sometimes twice if disease pressure is high. The first application is typically made in late July to reduce the impact of established *S. vaccinii* infections, while also managing for *T. minima* which becomes more problematic later in the season (Hildebrand et al., 2016). At this point in time the vegetative year fields have sprouted from the rhizomes and created a dense canopy which can result in extend periods of leaf wetness (see Chapter 2). Late July also corresponds with appearance of tip-dieback which occurs naturally when vertical vegetative growth from the shoot apical meristem stops (Smagula & DeGomez, 1987). If high amounts of leaf rust are observed in vegetative year fields and weather conditions are conducive for infection, a second application is made roughly 14 days after the first application; however, this can depend on if the grower has started harvesting their crop yield fields and has time to make a second application.

Fungicides that are currently registered for use on wild blueberry foliar diseases have single site modes of action and are systemic. Most growers use a tank mix of prothioconazole and benzovindiflupyr for managing foliar disease. Prothioconazole (Proline® 480 SC; Bayer Crop Science) is classified as a demethylation inhibitor (DMI) which inhibits C-14 demethylase during the synthesis of ergosterols in the cell membrane (Parker et al., 2011). Benzovindiflupyr (Aprovia®; Syngenta Crop Protection Inc) is classified as a succinate dehydrogenase inhibitor (SDHI) which is absorbed by growing fungal hyphae. It acts to inhibit fungal growth by blocking the activation site of succinate dehydrogenase, effectively stopping the electron transport chain process within the mitochondria of the fungal cell (Kuznetsov et al., 2018). It has also been observed as being highly effective at inhibiting spore germination when tested using different species of fungi (Juliatti et al., 2017; Chapter 3). In addition to these, pydiflumetofen was registered in 2022 for use on foliar diseases in the product Miravis® Neo. The label indicates that it controls blueberry leaf rust, and suppresses Valdensia and Sphaerulina leaf spot. Mefentrifluconazole became registered on wild blueberries in 2023 for use against Botrytis gray mold, Monilinia blight and Sphaerulina leaf spot. It is a DMI fungicide currently registered in Canada on other fruit crops such as apples and grapes and has shown high activity against foliar pathogens *Zymoseptoria tritici* (Septoria tritici blotch on wheat) and *Puccinia striiformis* f. sp. *tritici* (stripe rust on wheat) (Carmona et al., 2020; Ishii et al., 2021).

With only one fungicide application, occasionally two, being made on vegetative year fields, it is crucial for fungicides to provide protection from pathogens for an extended period of time. After systemic fungicides are applied, the concentration decreases over time

due to redistribution within the plant, chemical degradation, and dilution by actively growing plant tissues (Mondal et al., 2007). The efficacy of the fungicide seen at a plant or whole canopy scale can be dependent on distribution, mobility, and persistence in the plant. In some fungicide models that estimate efficacy in the field, the efficacy of the fungicide is a direct function of the residue and time since application (Arneson et al., 2002). To visualize the general trend of the relationship between residue and efficacy, when they are plotted against each other the typical curve takes the shape of a sigmoidal curve where efficacy is low when residue concentration is low, with efficacy increasing rapidly as residue increases, before eventually leveling off where increased residue does not change the efficacy (Caffi & Rossi, 2018). This trend has been observed in wild blueberry fields as foliar disease incidence increases with the duration of time since fungicide application (see Chapter 2).

4.1.2 Pesticide Residue Analysis

Today, gas chromatography coupled with mass spectrometry (GC-MS), or liquid chromatography coupled with mass spectrometry (LC-MS) are now the most widely used analytical methods and are consistently reported in the literature for assessing pesticide residues in various matrixes, including soil, water, plant, or animal samples (Grimalt & Dehouck, 2016; Heshmati et al., 2020; Silva, Lopez-Avila, & Pawliszyn, 2013). While GC-MS and LC-MS have been around for some time, the methods involved with extracting pesticides from plant material is relatively new (Anastassiades, 2003). Methods to extract pesticide residues have come to be known as the quick, easy, cheap, effective, rugged and safe (QuEChERS) method, which has become very popular for pesticide residue extractions (Anastassiades, 2003). Extraction can be followed by a solid phase extraction

(SPE) clean-up which is easily modifiable and have been successful at extracting a variety of analytes, from pesticides to antioxidants, in a variety of matrices (Bernardi et al., 2016; Chen et al., 2014; Sarraf, Beig-babaei, & Naji-Tabasi, 2020). Many studies use these analytical techniques to determine the fungicide residues found in consumer products such as fresh fruit and vegetables to protect the consumer from any residues that may exceed safe levels (Bakirci & Hişil, 2012). Other studies have used these techniques to assess the persistence of pesticides in the environment, or even in leaf tissues (Ryckaert et al., 2007).

Fungicide persistence and residues in wild blueberry flower and berry samples have been studied (Abbey, 2022). To extract fungicides in flowers and berries, the QuEChERS method followed by solid phase extraction (SPE) clean-up technique was used. When pydiflumetofen residues were analysed in wild blueberry flowers 24 hours after application, it was observed at a mean concentration of 5.69 $mg \cdot kg^{-1}$ of flowers. When fungicides were applied to protect the set berries 10 days after fungicide application, pydiflumetofen was observed at 0.29 $mg \cdot kg^{-1}$ of berries (Abbey, 2022). These results can be considered when estimating the fungicide persistence in wild blueberry leaves, however the amount of fungicide that reaches the leaves may be different since they are more horizontally positioned compared to the flowers or berries.

In the past, few studies have analysed the fungicide residues in leaf material for the same fungicides that are used on wild blueberries. Residues of prothioconazole and its major metabolite, prothioconazole-desthio, were assessed on wheat plants, with prothioconazole-desthio being found in greater concentrations than prothioconazole and being detected in plants at a concentration of 0.09 $mg \cdot kg^{-1}$ 21 days after application (Lin, Dong, & Hu, 2017). When prothioconazole and prothioconazole-desthio residues were

assessed on rice plants, it was again observed that prothioconazole-desthio had higher concentrations than prothioconazole and that the half life for prothioconazole-desthio was 6.6 to 10.1 days (Dong et al., 2019). For pydiflumentofen, residue analysis and dissipation studies have found that less than 5 $mg \cdot kg^{-1}$ was quantified in soybean plants 15 days after application and the half life in soybean plants was 3.6 and 5.7 days. For mefentrifluconazole, residue and dissipation rates were observed in rice. Here it was observed that the residues were 0.01 to 0.09 $mg \cdot kg^{-1}$ 21 days after application and had a half life of 11.0 and 16.6 days in rice straw (Zhang et al., 2023). No residue evaluation or dissipation studies have been conducted for benzovindiflupyr at this time, limiting our ability to estimate the dissipation in wild blueberry leaves.

The objective of the present study is to assess the residues and dissipation rates of various fungicides applied to wild blueberry fields for managing foliar diseases. With only one fungicide application, occasionally two, it is important to inform the industry of the duration that these chemicals are present in the leaves. This can provide wild blueberry growers with the information to determine if or when a second fungicide application is required.

4.2 Materials and Methods

4.2.1 Experimental Design

The field trial was conducted in a commercial wild blueberry in Debert, Nova Scotia (coordinates = 45°26’35.65 N, 63°27’5.69 W) during the summer of 2022. The field was in the vegetative year of the production cycle when the trial was initiated. The trial was set up as a randomized complete block design (RCBD), with 4 replicates and 5 treatments. The five treatments were: (1) untreated control, (2) mefentrifluconazole (Cevya®), (3)

pydiflumetofen (Miravis® Bold), (4) prothioconazole (Proline® 480 SC), and (5) benzovindiflupyr (Aprovia®). Treatment plots were set up in the field using plastic plot markers, with plots being 4 by 4m in size and 2 m wide alleyways between plots. Plastic treatment tags were attached to the plot markers to indicate the treatment. The field had a Watchdog® weather station model 2700 (Aurora, IL, USA) to monitor air temperature, relative humidity, and leaf wetness. Data were logged every 30 minute for the duration of the trial.

4.2.2 Fungicide Application and Sample Collection

Fungicides were applied only once at the typical application time, which is at the first sign of leaf rust and tip-dieback. Leaf rust was identified by its distinct yellow pustules on the abaxial leaf surface which also corresponded with tip-dieback stage. Fungicides were applied on July 27, 2022. The weather at the time of application was 25.9 ℃, 76% RH, 3.4 $km \cdot h^{-1}$ windspeed and sunny. Fungicides were applied at their label rates (Table 4.1) with a water volume of 210 $L \cdot ha^{-1}$. Active Plus™ (WinField® United) was added as an adjuvant at a rate of 370 $mL \cdot ha^{-1}$ which is common industry practice for foliar fungicide application. Fungicides in their commercially available form were mixed with tap water in 2 L spray bottles to their specified concentration and shaken in the bottles to mix effectively. Application was made using a Bellspray Inc. Model GS carbon dioxide pressurized hand sprayer 30 cm above the crop canopy at 32 PSI with a 2 m wide spray pattern fit with four Teejet Visiflow 80-02VS nozzles (Figure 2.1). The nozzle discharge rate was 10 $mL \cdot s^{-1}$ and the application ground speed was 1 $m \cdot s^{-1}$. To ensure accurate application rate to the plots, the amount of liquid emitted from the nozzles was measured and used to calculate

walking speed. A metronome was used to maintain constant walking speed. Two side by side passes were made to evenly cover the entire 4 by 4 m plot.

Table 4.1 Fungicide treatments, active ingredients and application rate used in the field trial in Debert. All fungicides were applied in accordance with their label rates at a water volume of 210 $L \cdot ha^{-1}$. Year registered refers to the year these fungicides were registered on wild blueberries in Canada.

Treatment	Active Ingredient	Year Registered*	Product Application Rate ($L \cdot ha^{-1}$)	Amount of Active Ingredient ($g \cdot ha^{-1}$)
Untreated control				
Cevya®	mefentrifluconazole	NR	0.25	100
Miravis® Bold	pydiflumetofen	2022	1.0	200
Proline® 480 SC	prothioconazole	2011	0.4	192
Aprovia®	benzovindiflupyr	2016	0.75	75

* NR = Not registered

Plant samples were collected 2 hrs after application and then 7, 14, and 30 days after application (DAA). At each sampling date, 30 stems were randomly removed from each plot with the leaves remaining on the stems. Thirty stems per plot were used to ensure there would be at least 5 g of leaves for residue extractions. The plant samples were cut at the soil surface, put in plastic bags labeled with replicate and treatment, and placed into a cooler with ice packs until samples arrived at the lab. On the same day as sampling, bags of samples were placed in the freezer (-18°C).

4.2.3 Fungicide Residue Analysis Using QuEChERS Method, Detection by GC-MS and LC-MS

4.2.3.1 Chemicals and standards

External standard of pydiflumetofen and benzovindiflupyr were obtained from Syngenta Canada (Greensboro, NC), mefentrifluconazole was purchased from Chem Service, Inc. (West Chester, PA), prothioconazole and prothioconazole-desthio were obtained from Bayer Crop Science (Kansas City, USA) and the internal standard triphenyl

phosphate (TPP) was obtained from Acros Organics (Germany). Analytical grade toluene and methanol (Fisher Scientific, ON, Canada) were used. Anhydrous magnesium sulphate ($MgSO_4$) (Sigma-Aldrich), and primary-secondary amine (PSA) (Cole Parmer, USA) were used during the extraction and clean up procedures.

4.2.3.2 Extraction Procedure and Clean-up

Sample preparation was completed using the QuEChERS method described by Heshmati et al., (2020) with modification from Abbey (2022). Samples of leaves were removed from the stem and homogenized using a mortar and pestle with the addition of liquid nitrogen (Figure 1A). For each sample, 5 g of homogenized leaves were weighted into a 50 mL centrifuge tube and 10 g deionized water was added to make the 15 g starting samples. Then, 15 mL of 1% acetic acid in acetonitrile was added. TPP, 1.25 mL of 10 µg mL^{-1} was added to the centrifuge tube and shaken vigorously by hand. A buffer-salt mixture of 6 g MgSO4 and 1.5 g NaOAc was also added to the centrifuge tube and shaken vigorously by hand again. The tube was then centrifuged for 7 min at 4500 rcf and 0 °C (Figure 1B). After centrifuging, 4 mL of the supernatant layer was transferred to a 15 mL centrifuge tube for dispersive solid phase extraction (SPE) clean up.

SPE extraction was done by removing 4 mL of extract from the 50 mL centrifuge tube and adding to a 15 mL centrifuge tube containing 600 mg $MgSO_4$ (150 mg·mL^{-1} extract), 200 mg PSA (50 mg·mL^{-1} extract). The mixture was shaken and then centrifuged for 7 min at 4500 rcf and 0 °C (Figure 1C). After centrifuging, 1 mL of the supernatant layer was removed and placed in a 15 mL centrifuge tube. The extract was evaporated under a gentle stream of nitrogen at 30 °C and reconstituted using 10 mL of toluene. The extract was reconstituted using a high volume (10 mL) to dilute the sample and reduce the

concentration of green pigments. Graphitized carbon black (GCB) was not used due to the potential interference with analytes. Samples that were prepared for analysis using LC-MS were reconstituted using 10 mL of toluene:methanol (1:1) mixture. Samples were passed through a 0.45 µm nylon filter before adding to vials with 250 µL inserts for analysis on GC-MS and LC-MS (Figure 1D).

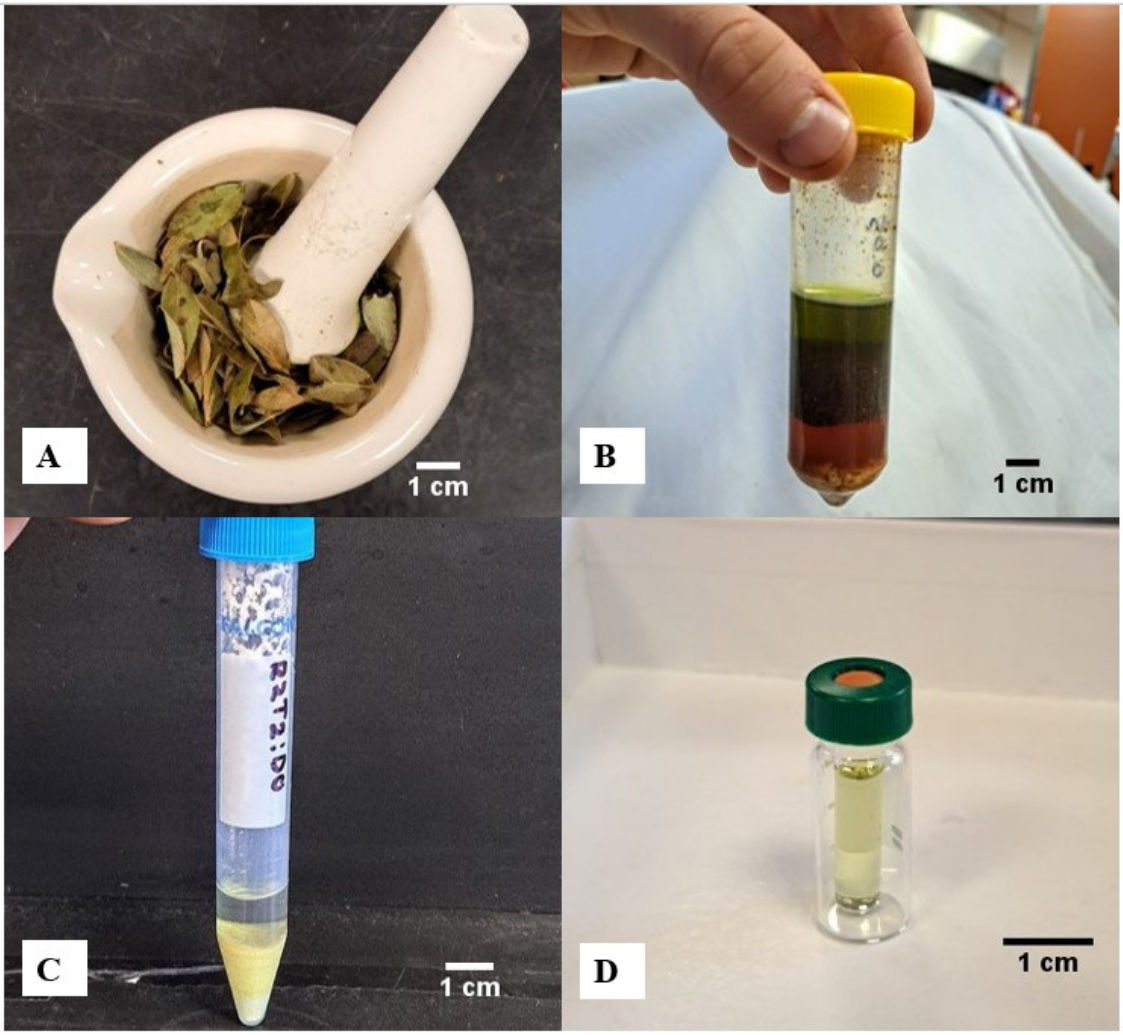

Figure 4.1. (A) A mortar and pestle was used to homogenize leaf samples, adding liquid nitrogen when required to easily grind samples, (B) after the additions of acetonitrile, MgSO4 and NAOAc and centrifuging, the solution separated into 4 distinct layers with the supernatant layer being removed for solid phase extraction, (C) after solid phase extraction using $MgSO_4$ and PSA the supernatant was removed again, and (D) passed through a 0.45 µm filter before adding to a 250 µL insert in a GC-MS vial.

4.2.3.3 Gas Chromatographic, Liquid Chromatographic, and Mass Spectral Conditions

GC-MS at the Dalhousie University Agricultural Campus (Truro, NS) was used to complete residue testing for benzovindiflupyr and pydiflumetofen, and prothioconazole, desthio-prothioconazole and mefentrifluconazole residues were tested at Trent University Water Quality Center (Peterborough, ON). Different analytes were tested on different machines since prothioconazole and desthio-prothioconazole are unable to be accurately assessed using GC, and mefentrifluconazole has not been documented as being successfully detected using GC (SANCO/12745/2013). Similar GC conditions were used as described in Chen et al. (2014). The instrument used was a Scion 456A GC-triple-quadrupole mass spectrometer, equipped with a Bruker autosampler (Bruker, Scion Instrument, Amundsenweg, Netherlands) which made 2 uL (1:20 split) injections. Separation was achieved on a 30 m x 0.25 mm x 0.5 μm capillary column. Helium (99.9% purity) at a flow rate of 1.2 $mL \cdot min^{-1}$ was the carrier gas. The oven temperature program was set to start at 80 °C (hold for 1 min), then increasing to 180 °C at 25 $°C \cdot min^{-1}$ (holding for 1 min), and then increasing to 310 °C at 10 $°C \cdot min^{-1}$ (holding for 5 min). The ion source and MS transfer line was set at 280 °C. Electron ionization of 70 eV with select ion monitoring (SIM) mode detecting 2-3 ions for each tested analyte was used.

The LC-MS/MS analysis was completed for prothioconazole, prothioconazole-desthio and mefentrifluconazole using an Agilent 1100 Series LC and autosampler (Mississauga, ON Canada), located at the Water Quality Center at Trent University (Peterborough, ON). At the Water Quality Center, 100 uL samples of extracted matrix were spiked with 10 uL of 1 $ug \cdot mL^{-1}$ isotopically labelled internal standard (atrazine-D5 in methanol). Samples were evaporated using nitrogen gas and reconstituted in 100 uL of

methanol. Once prepared for the LC-MS/MS instrument, samples were separated in a Thermo Acclaim RS LC 120 C18 column (2.2 µm, 2.1 x 50 mm) with a C18 guard cartridge, using a gradient elution and 0.1% formic acid in high purity 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 minutes, returned to initial conditions after 0.5 minutes and re-equilibrated for 3.5 minutes, resulting in a total run time of 8.5 minutes. Injection volume was 20 mL and flow rate was 0.550 $mL \cdot min^{-1}$.

4.2.3.4 Method Validation

Method validation was performed to ensure that the extraction and clean-up methods are suitable for accurately assessing the analytes in the specific matrix. Method validation was only done for pydiflumetofen and benzovindiflupyr fungicides because they were able to be analysed within the Dalhousie Agricultural Campus, however, the method validation can be extended to the other analytes as has been done previously. The guidelines established by the European Union for analytical quality control and procedures for method validation for pesticide residue testing were followed (SANTE/11312/2021). In the guidelines, calibration linearity, accurate pesticide recovery, limit of quantification (LOQ), limit of detection (LOD), precision, and matrix effect are described.

The linearity of the calibration curve was determined by analyzing six calibration concentrations at 0.005, 0.01, 0.05, 0.2, 0.5, and 1.0 $ug \cdot mL^{-1}$ (n=3) in pure toluene solvent with internal standard and calculating the R^2. This was repeated using blank extracted leaf matrix. Based on these calibration curves, the limit of detection (LOD) (LOD = 3.3 x S/N x 0.5) and limit of quantification (LOQ) (LOQ = 10 x S/N x 0.5) were calculated using the

signal to noise ratio (S/N) where the signal is the standard deviation of the calibration curve and the noise is the slope of the curve.

To measure the recovery of fungicides using this method, blank samples of wild blueberry leaves were spiked at 0.1, 0.5 and 1.0 $mg \cdot kg^{-1}$ and then extracted using the methods mentioned above. Recovery was assessed using six replicates for each concentration, with each concentration being completed on a different day. The average and relative standard deviation (RSD%) were calculated. Using the average and RSD% the repeatability and inter-day precision, was assessed. Repeatability was analysed using RSD% from six replicates within the day, and inter-day precision was assessed using the RSD% for the three extraction days.

Matrix effect was assessed by extracting a blank sample and adding fungicide to the matrix at 0.005, 0.01, 0.05, 0.2, 0.5 and 1.0 1.0 $ug \cdot mL^{-1}$ (n=3) to make a matrix calibration curve. Matrix effect (ME) was calculated as (1 – (slope of $curve_{solvent}$/slope of $curve_{matrix}$) x 100%.

4.2.4 Statistical Analysis

The degradation kinetics for each fungicide was assessed by plotting the residue ($mg \cdot kg^{-1}$) against time. Curves the had a high correlation coefficient (R^2) was used to describe the degradation kinetics. Plotting and regression correlation was done using SigmaPlot (version 12.0, software Inc., San Jose, California, US). The degradation of these fungicides followed the first-order rate kinetics, where it can be characterized by the equation $C_t = C_0\, e^{-kt}$, where C_0 and C_t are the fungicide concentration ($mg \cdot kg^{-1}$) 2 hrs after application and on day t, respectively, and k is the degradation rate constant (Heshmati et al., 2020).

4.3 Results

4.3.1 Method Validation

Using the matrix-matched calibration curve, the LOD and LOQ for pydiflumetofen and benzovindiflupyr were calculated (Table 4.2). A significant matrix effect was observed (> 20%) for both fungicides, when the slope of the matrix-matched and solvent calibration curve were compared (Table 4.2). The signal of both compounds was found to be suppressed (negative ME) (Table 4.2). Linearity of the matrix-matched calibration curve was assessed using concentrations 0.005 to 1.0 $\mu g \cdot mL^{-1}$ and provided high correlation coefficients for both pydiflumetofen and benzovindiflupyr (Table 4.2). The regression equation derived from the matrix matched calibration curve was used to calculate the residues in test samples.

Table 4.2 The retention time, regression coefficient, limit of detection and limit of quantification calculated using the respective analytical standards in solvent only. The matrix effect determined using blank extracted matrix spiked at six concentrations.

	Retention Time (min)	R^2	LOD ($mg \cdot kg^{-1}$)	LOQ ($mg \cdot kg^{-1}$)	Matrix effect (%)
Pydiflumetofen*	17.0	0.997	0.011	0.034	-45.8
Benzovindiflupyr*	18.2	0.997	0.012	0.037	-59.7
Prothioconazole**	3.79	0.999	-	-	-
Prothioconazole-desthio**	3.65	0.999	0.001	0.0028	-
Mefentrifluconazole**	3.73	0.996	0.003	0.0078	-

*analysis was done using GC-MS
**analysis was done using LC-MS

The mean recovery for pydiflumetofen ranged from 91.4 to 125.8%, 81.8 to 90.8%, and 85.7 to 118.7% for spiked concentrations 0.1, 0.5 and 1.0 $mg \cdot kg^{-1}$, respectively (Table 4.3). For benzovindiflupyr, the mean recovery ranged from 52.8 to 90.9%, 80.3 to 113.5%, and 72.9 to 121.3% for spiked concentrations 0.1, 0.5 and 1.0 $mg \cdot kg^{-1}$, respectively (Table 4.3). The extraction method precision was calculated using the relative standard deviation

(RSD %) which ranged from 4.1 to 13.1% for the intra-day precision for pydiflumetofen, and from 0.9 to 25.3% for benzovindiflupyr (Table 4.3). The RSD % was high for the lowest spiked concentration, this should be considered when assessing the residue analysis results.

Table 4.3 The mean recovery (%) from blank samples spiked at three concentrations and precision calculated using the relative standard deviation (RSD %) for the intra-day (repeatability) and inter-day (intermediate). Means calculated using six replicates.

	Recovery (%) (n=6)			**Precision (RSD%) (n=6)**			
	Concentration ($mg \cdot kg^{-1}$)			**Concentration ($mg \cdot kg^{-1}$)**			
				Intraday			**Inter-day**
	0.1	**0.5**	**1.0**	**0.1**	**0.5**	**1.0**	
Pydiflumetofen	106.0	86.0	101.8	12.4	4.1	13.1	13.9
Benzovindiflupyr	69.2	93.2	99.2	26.5	0.9	20.7	23.9

4.3.1 Fungicide Residues in Wild Blueberry Leaves

Once the method validation procedure was completed, the same extraction methods were used to evaluate the persistence of fungicides in wild blueberry leaves 2 hours after application and then 7, 14 and 30 DAA. Using the fungicide residues ($mg \cdot kg^{-1}$) detected at the various sampling dates, the dissipation curve using the first-order kinetics equation could be generated (Figure 4.2). Prothioconazole was not detected in any of the samples that were analysed using LC-MS. Two hours after application, the initial fungicide concentrations for pydiflumetofen, benzovindiflupyr, prothioconazole-desthio and mefentrifluconazole was 18.1, 9.01, 1.17 and 2.68 $mg \cdot kg^{-1}$, respectively (Figure 4.2). Variability was also observed between fungicides when examining the proportion of the initial fungicide residues detected 30 DAA (Figure 4.2). After 30 days subjected to field conditions, residues for pydiflumetofen, benzovindiflupyr, prothioconazole-desthio and

mefentrifluconazole were 33.8, 18.4, 33.3 and 50.7% of their initial detected concentration, respectively (Figure 4.2). For pydiflumetofen, prothioconazole-desthio and mefentrifluconazole, the concentration detected 7 DAA was greater than the initial residue detected 2 hours after application.

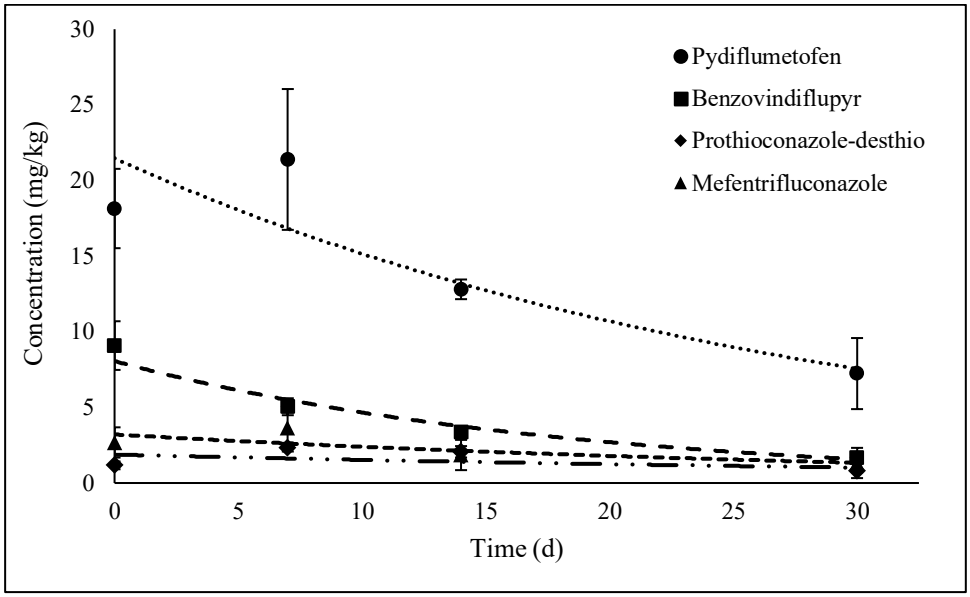

Figure 4.2. The dissipation of pydiflumetofen, benzovindiflupyr, prothioconazole, prothioconazole-desthio and mefentrifluconazole in wild blueberry leaves under field conditions at various sampling dates (n=4). The trend line models the first-order kinetics equation.

Using the residues detected at each sampling date and the first-order reaction kinetics equation, the half-life of the fungicides was calculated (Table 4.4). The half-life of pydiflumetofen, benzovindiflupyr, prothioconazole-desthio and mefentrifluconazole were 19.8, 12.6, 34.7, and 23.9 days, respectively (Table 4.4). It can be noted that the half-life for prothioconazole-desthio was 31.1% greater than all other fungicides.

Table 4.4 Using the first-order kinetics, the corresponding regression coefficients (R^2), decay coefficient (k) and first-order reaction equation were determined. Using this equation, the half life was calculated.

	R^2	k	Equation ($Ct=Coe^{-kt}$)	Half-life (days)
Pydiflumetofen	0.73	0.035	$y=21.475e^{-0.035t}$	19.8
Benzovindiflupyr	0.97	0.055	$y=8.052e^{-0.055t}$	12.6
Prothioconazole	-	-	-	-
Prothioconazole-desthio	0.12	0.02	$y=1.8727e^{-0.02t}$	34.7
Mefentrifluconazole	0.54	0.029	$y=3.209e^{-0.029t}$	23.9

4.3 Discussion

High variability of initial concentrations was observed between fungicides detected in leaf samples collected 2 hours after application, this may be a result of the quantity of active ingredient applied or the analytical methods used. For example, 2 hours after application the quantity of pydiflumetofen detected was 50.1% greater than that of benzovindiflupyr. This is most likely due to the differences in grams of active ingredient that is applied per hectare at the rates used in this study. The rates used in the study were the recommended label rates typically used on wild blueberries, where 200 grams of pydiflumetofen is applied per hectare, compared to 75 grams per hectare of benzovindiflupyr (Table 4.1). The differences in amount of active ingredient applied to the field is the main reason for not statistically analyzing the differences in fungicide concentration at each sampling date. The variability noted 2 hours after application could also be a result of analytical methods used (ie. GC-MS or LC-MS). For example, pydiflumetofen, benzovindiflupyr were analysed using GC-MS, with the extraction methodologies validated, were found in much greater concentrations than prothioconazole-desthio and mefentrifluconazole which were analysed using LC-MS and did not have the extraction methods validated for these compounds. This is a drawback of using two types

of analytical methods, however two analytical methods were used due to equipment availability.

Prothioconazole was not detected in extracted leaf samples at any of the sampling dates. This was a surprising result considering other studies have successfully detected prothioconazole in other plants such as rice and wheat (Lin, Dong, & Hu, 2017; Liang, Chen & Hu, 2021). Liang, Chen & Hu (2021) outline their extraction methods, which use very similar reagents and solvents as compared to the present study. Liang, Chen & Hu (2021) even used graphitized carbon black (GCB), which has been documented as being responsible for precipitating pesticides out of samples resulting in decreased recovery during extraction (Lu et al., 2021). One primary difference between these studies and the present one is the completion of the method validation step, more specifically, the recovery study and matrix effect. Completing these method validation components can help adjust the solvent concentrations and overall extraction methods to improve recoverability. These components of the method validation step were unable to be completed due to the barrier of having different lab complete the analysis and the cost of analysis.

For pydiflumetofen, prothioconazole-desthio and mefentrifluconazole, it was observed that the concentration detected 7 DAA was greater than the initial residue detected 2 hours after application. For prothioconazole-desthio, this is expected due to the conversion of prothioconazole to prothioconazole-desthio which has been observed to occur over 8 days. When the ratio of prothioconazole and prothioconazole-desthio were compared in wheat plants 2, 4 and 8 days after application, the ratio was 1:0.34, 1:0.65 and 1:1.15, respectively. This provides a rough estimate for the timescale required for prothioconazole to convert to prothioconazole-desthio (Lehoczki-Krsjak et al., 2013). For

pydiflumetofen, the increase in concentration from 0 to 7 DAA could be described by the large standard error associated with the 7 DAA mean concentration (Figure 4.2).

As for mefentrifluconazole, the reason for increased concentration from 0 to 7 DAA can be hypothesized as being due to the impact of cuticle waxes on the extraction of fungicides from the leaf. At 2 hours after application, the fungicide may have bound to the cuticle waxes and was not extracted into solution during the QuEChERS extraction procedure. Mefentrifluconazole is moderately lipophilic (K_{ow} 3.4) which means it can be difficult to separate from the cuticular wax that covers the adaxial leaf surface (Oellig, 2016; Skrzydeł, Borowska-wykręt, & Kwiatkowska, 2021). In comparison, 7 DAA the fungicide may have passed through the cuticle layer and into the mesophyll layer where the extraction procedure effectively removed it. If lipophilicity is the phenomenon causing the increase in concentration from 0 to 7 DAA, this would have also been expected with benzovindiflupyr, which has a K_{ow} of approximately 4.3 (University of Hertfordshire, 2023).

Although limited studies have quantified benzovindiflupyr residues in plants, the residue analysis has been completed in wheat and soybean plants, both of which had similar residue concentrations to that found in wild blueberry leaves (FAO, 2016; EFSA, 2015). For indoor grown wheat, benzovindiflupyr was applied twice at 120 grams per hectare and was quantified in plants at 8.1 $mg \cdot kg^{-1}$ 40 DAA (FAO, 2016). Benzovindiflupyr was also quantified in indoor grown soybeans after two applications at 120 grams per hectare. Here, benzovindiflupyr was found at 14 mg/kg when assessed 30 DAA. In both of these studies the major residue (>97%) was found to be benzovindiflupyr and not a metabolite (EFSA,

2015). The double application and the higher rate should be noted here in comparison to a single application at 75 grams per hectare of benzovindiflupyr in the present study.

Benzovindiflupyr persistence and breakdown in soil is quite different than that observed in plants. After application to soils at 530 grams per hectare, the half-life was documented as being up to 1,216 days. In addition, the parent compound and its metabolites, pyrazole acid and SYN545720, were found in the plant tissue of leafy greens, tubers and cereals the year after application (EFSA, 2021). The effect of light on benzovindiflupyr persistence in soil has also been studied, where the presence of light reduced the half-life by 50% (Davies et al., 2013). The effect of non-UV light on benzovindiflupyr degradation is hypothesized as being a result of the effect of light on microbial communities, pH and photolysis (Davies et al., 2013).

Pydiflumetofen is a broad-spectrum fungicide with activity on a variety of pathogens, however, limited studies have examined the dissipation in plants. Pydiflumetofen was applied to grapes and soil at the Institute of Plant Protection in China to determine the half-life in both matrices (Wu et al., 2020). The half-life was observed to be 11 and 14 days in grapes and soil, respectively (Wu et al., 2020). In comparison, the half-life in soybean plants was found to be between 3.6 and 5.7 days, approximately 77% shorter than that of the present study. Factors that could influence these differences are the crop growth stage, climatic conditions and crop morphology.

Despite limited degradation studies due to the recent registration of mefentrifluconazole in certain parts of the world, the degradation in rice and cucumbers has been evaluated (Tesh et al., 2019). The half-life for mefentrifluconazole in rice straw was observed to be between 11.0 and 16.6 days (Zhang et al., 2023). Contrary those results,

when the half-life was evaluated in cucumber it was found to be 4.0 days (Li et al., 2021). The discrepancy here could be a result of the crop growth stage at application timing, where the crop growth stage when applied to rice was not indicated, but was applied to cucumber when the fruit was mid-sized. The dilution of fungicide due to increasing fruit size can strongly impact the calculated degradation rates of pesticides (Miles et al., 1963). In comparison, the half-life for mefentrifluconazole observed in the present study was 23.9 days.

Prothioconazole-desthio residues in rice plants have been observed as having a half life of 2.5 to 10.1 days when applied at 315 g a.i.·ha^{-1} (Dong et al., 2019). Due to the rapid conversion of prothioconazole to prothioconazole-desthio, prothioconazole was not detected in the sampled rice plants (Dong et al., 2019). Most studies focus on the dissipation of the metabolite, prothioconazole-desthio, which is more efficacious and more toxic to nontarget organisms (Tian et al., 2022; C. Wu et al., 2022). When the dissipation of prothioconazole-desthio was studied in wheat fields, it was observed that residues detected in the grain were less than 0.01 mg·kg^{-1}, and between 0.01 and 0.49 mg·kg^{-1} in wheat straw (Liang, Chen & Hu, 2021). Sun et al. (2018) also studied prothioconazole and its metabolites degradation in wheat straw when applied at 300 g a.i.·ha^{-1}, and they observed that prothioconazole had a half-life of 3.2 days, whereas prothioconazole-desthio had a half-life of 5.0 days. In comparison to the results of the present study, prothioconazole-desthio was observed to have a half-life of 34.7 days, much longer than any previous studies.

Many factors beyond the amount of active ingredient applied to the field such as fungicide stability, volatilization, photodegradation, microbial degradation, weather, crop

morphology, and crop growth stage can influence the dissipation and persistence of pesticides (Chai, Mohd-Tahir, & Hansen, 2009). The role these factors play in pesticide degradation has been widely studied and the degree to which they impact degradation has been noted as pesticide dependent (Xi, Li, & Xia, 2022). And with that, limited studies have analysed the impact of these factors on the degradation of fungicides, let alone the fungicides evaluated in the present study. Despite this, the concepts that have been observed when assessing other pesticides can be hypothesized as explanations for the trends that were observed in the present study.

Bromilow, Evans, & Nicholls (1999) studied the degradation of five triazole fungicides in soil under different temperature, soil moisture and soil type. They observed that degradation of triazoles was slower at lower temperature and did not notice any other consistent trends regarding soil moisture and soil type. The differences seen in degradation at different temperatures may be due to the role of microbial degradation. The degradation rates observed in soil matrix may differ when comparing to the degradation in plants because of the differences in microbial communities (Hamed et al., 2022). Microbial degradation occurs when microorganisms assimilate carbon for energy or nutrients from the pesticide chemical by hydrolysis or other reactions to benefit their growth (Karns et al., 1986).

The microbial degradation of phosmet, an insecticide used on wild blueberries for managing blueberry maggot, spanworm larvae and flea beetles, has been studied after being applied to blueberry fruit (Crowe et al., 2007). Here the degradation of phosmet was slow 24 hours after inoculation with predominant bacterial cultures, but then increased after 48 hours, and increased growth of the bacterial culture followed. After 7 days, the insecticide

concentration had decreased by 33.8%. Similarly, the effect of chlorothalonil and prothioconazole fungicides on the soil microbiome has been studied in wild blueberry fields (Llyod, Percival & Yurgel, 2021). Fungal species richness was lower for soils treated with fungicides, compared to that of untreated. On the other hand, an increase in bacterial enzymes known to be associated with the decomposition of halogen containing organic molecules. Chlorothalonil and prothioconazole both contain halogen atoms, chlorine. It can be hypothesized that the increase in these bacterial enzymes is related to the microbial degradation of fungicides (Lloyd, Percival & Yurgel, 2021). Although these studies highlight the impact that microbes can have on pesticide degradation, every chemical will be degraded differently and should be considered when being applied to the present study. Endophytes and other microorganisms are present on and in plants, however the quantity and diversity of the microbiome on wild blueberry leaves is unknown.

Weather station datum collected from July 27, 2022 to August 27, 2022 was recorded at the Wild Blueberry Research Center in Debert, NS, where the trial was located (Figure A-3). Using the collected weather data to try an explain reasons for the dissipation patterns can be difficult due to the natural daily variation in the conditions. Despite this, other studies in controlled environments or focussed on environmental conditions have been conducted to try and explain the influence of the environment of the degradation of pesticides in plants.

Climatic variables such as volatilization and photodegradation can influence pesticide degradation when sprayed in the field. Pesticide volatilization occurs after the pesticides is sprayed and have been distributed onto the plant surface (Jacobsen, Fantke, & Trapp, 2015). Using models derived from field or controlled environment studies, the

proportion of pesticide lost to volatilization has been estimated at ranging from a few percent to 30% (Van Den Berg et al., 1999). Many pesticides have low vapour pressures, especially when combined with a surfactant which can reduce the proportion volatilized (Jacobsen et al., 2015). Pesticides can also degrade as a result of exposure to direct sunlight on the leaf surface. Here, the photons excite pesticide molecules and result in the organic reactions of a pesticides functional groups or the photolysis of bonds (Katagi, 2004). When the degradation of triazoles (cyproconazole, hexaconazole, penconazole, propiconazole, tebuconazole) was studied after application to peaches, it was shown that photodegradation strongly influences fungicide degradation (Angioni et al., 2003). The corresponding half-life of the fungicides was found to be between 3 and 14 days, but when the epicuticular waxes were removed from the peaches, the half-life dropped to between 2 and 2.5 days.

The role of plant morphology, in particular the waxy leaf surface composition, can influence the photodegradation of pesticides (Xi et al., 2022). Angioni et al. (2004) studied the effect of fruit and vegetable epicuticular waxes on the insecticide rotenone. The degradation rate and pathway were found to differ depending on the plant sample. Within 3 hours, the quantity degraded from the plant surface varied by up to 98%. The epicuticular waxes found on apple and pear increased the degradation rate of rotenone by up to 90%, whereas plum and nectarine epicuticular waxes decreased the degradation rate by up to 62% (Angioni et al., 2004). Cuticular waxes can prevent and slow photodegradation on the leaf surface by screening the light wavelengths, and by reducing the amount of photons (Anderson et al., 2016). On the other hand, photoreduction of the pesticide can occur as a result of photoexcited hydrogens from the wax, or cuticular waxes can also act as hydroxyl

group donors which can aid the degradation on the surface (Ter Halle, Drncova, & Richard, 2006; Xi et al., 2022).

Another potential reason for the reduction in fungicide concentration over time can be attributed to the increase in plant growth which can dilute the fungicide concentration (Karthika & Muraleedharan, 2009). Although fungicides were applied at tip-dieback stage, when the vertical vegetative growth from the shoot apical meristem stops, the size and mass of the leaves may increase after this point. In peaches, the dilution of select triazole fungicides (cyproconazole, hexaconazole, penconazole, propiconazole, tebuconazole) was documented as decreasing in concentration, with one of the major influences being the increase in peach mass, which influenced the concentration (mg/kg) (Angioni et al., 2003). The magnitude of concentration decrease varies from plant to plant and is dependent on the growth stage, but is has been estimated that for some plants, dilution can lead to a 60 to 80% decrease in fungicide concentration, possibly more influential on concentration than losses due to volatilization or degradation (Hopkins et al., 1952; Miles et al., 1963).

4.4 Conclusion

The results of this study outline the use of the QuEChERS extraction methods for detecting fungicide residues in wild blueberry leaves. The recovery and matrix effect were calculated and used to validate the extraction methods. Although differences in fungicide concentration were noted between the two analytical techniques, GC-MS and LC-MS, the calculated half-life can be used to compare the persistence of fungicides since half-lives incorporate the initial concentration and dissipation rate.

In general, fungicide dissipation followed first-order reaction kinetics, however some fungicides were detected in greater concentrations 7 DAA, compared to 2 hours after.

Thirty days after application, fungicides were still detected in blueberry leaves, indicating their potential to still be providing management of diseases. This is beneficial to wild blueberry growers since only one application, sometimes two are made for managing foliar diseases. This is especially important, since a fungicide application is made approximately at the end of July, while pathogens remain persistent and active until late September. The persistence of fungicides in leaf tissues can help inform the industry of whether one or two fungicide applications should be made for foliar disease management.

CHAPTER 5
CONCLUSION

5 Overview

Plant pathogens are one of the several biological challenges that wild blueberry growers face in eastern Canada and US. Foliar diseases including blueberry leaf rust, Sphaerulina leaf spot and Valdensia leaf spot, caused by *Thekopsora minima*, *Sphaerulina vaccinii* and *Valdensia heterodoxa*, respectively, cause significant yield reductions. For years, chlorothalonil used in conjunction with prothioconazole had been the fungicide combination of choice for managing foliar diseases. However, chlorothalonil was deregistered for use on wild blueberries in Canada in 2022. Due to its deregistration, a comparative assessment of alternative fungicide options is required to help wild blueberry growers manage foliar diseases and receive an optimum return on investment for their disease management strategies.

This study explored the effect of various fungicides and fungicide combinations on foliar diseases and the corresponding impact of foliar diseases on the physiology of the wild blueberry plant. To do this, fungicides were applied to plots within a field and were assessed 30 and 60 days after application for percent diseased leaves per stem, leaf number and NDVI. As the plants prepared for dormancy, floral bud number per stem was assessed. In the following year, as berries began forming, yield components and then yield were measured. By making assessments throughout the cropping cycle, the effect of fungicides on foliar disease management and plant physiological responses during the entire cropping cycle were measured.

The results of this portion of the study found that foliar fungicides are an effective disease management option for wild blueberry growers. Foliar diseases drastically increased from 30 days after application to 60 days, and rust became the dominant disease, outlining its virulence. The reasoning for an increase in foliar diseases could be a result of weather conditions and the polycyclic nature of *T. minima*, or as it was noted in the fungicide persistence testing, it could be a result of decreases fungicide concentration in the leaf tissues. On average across assessment dates and locations, fungicide treatment prothioconazole with benzovindiflupyr reduced disease and maintained leaf number greater than other treatments. Both fungicides are currently registered for use on wild blueberries. Despite the reduction in diseased leaves and increased leaf number observed in plots treated with prothioconazole with benzovindiflupyr, this treatment did not have increased floral bud number or yield. One potential explanation for this trend could be that wild blueberries draw on their extensive rhizome system, which acts as a carbon source, to make up for a lack of carbon assimilation as a result of leaf diseases. Alternatively, the long two-year cropping cycle and high variability (i.e. species, phenotype, plant density) found in wild blueberry fields could reduce the influence of a single fungicide application. Results also indicated that new fungicides which are not registered for use on wild blueberries, such as mefentrifluconazole, pydiflumetofen and folpet provided some protection against diseases, however further research investigating their use in combination with other fungicides is required.

The present study also examined the in-vitro sensitivity of foliar pathogens to fungicides using a collection of fungi isolated from across Nova Scotia. Fungicide resistance has been documented for other pathogens on wild blueberries, so examining

foliar pathogen sensitivity can provide a temporal reference point for fungicides that have been used to manage foliar pathogens for years, such as prothioconazole, and a baseline sensitivity assessment for fungicides that are not yet registered for use. Here, the fungicide sensitivity of *S. vaccinii* was measured using the light absorbance microplate assay method, and fungicide sensitivity of *T. minima* was measured using urediniospore germination assay. *S. vaccinii* isolates were highly sensitive to prothioconazole-desthio compared to other fungicides and especially compared to its parent compound prothioconazole. *S. vaccinii* sensitivity results did not appear to be impacted by the location of the isolate. When the sensitivity *S. vaccinii* isolates collected in 2009 were compared to those collected in 2022 for prothioconazole-desthio, the 2022 isolates did not appear to have significantly less sensitivity to compared to the 2009 isolates. This could be an indication that minimal development of resistance has occurred since prothioconazole began being used for wild blueberry foliar disease management roughly 10 years ago. Regarding *T. minima*, isolates were most sensitive to benzovindiflupyr compared to all other fungicides. The DMI fungicides prothioconazole, prothioconazole-desthio and mefentrifluconazole did not appear to greatly inhibit spore germination. *T. minima* sensitivity results should be considered in the context that urediniospore germination is only one small step in the fungal life cycle. Lastly, the methods outlined here can be used to continuously monitor these fungal populations for the development of resistance to fungicides being used by the wild blueberry industry. In other horticultural crop industries, consistent fungicide resistance monitoring has become a key component for ensuring that fungicides continue to be an efficacious disease management tool.

In addition to fungicide efficacy and sensitivity, the persistence of fungicide active ingredients in wild blueberry leaves was evaluated. Determining the persistence within the leaf tissue can help determine the duration of protection that fungicides may provide. For this portion of the study, fungicides were sprayed in the field at the typical application timing for foliar disease management, and the plant samples were collected 2 hours after application, and then again 7, 14 and 30 days after. Extraction of fungicides from leaf tissue was completed using a quick, easy, cheap, effective, rugged and safe (QuEChERS) followed by solid phase extraction (SPE). Fungicides pydiflumetofen and benzovindiflupyr were assessed using gas chromatography-tandem mass spectrometry (GC-MS) while, prothioconazole, prothioconazole-desthio and mefentrifluconazole were assessed using liquid chromatography-tandem mass spectrometry (LC-MS). Results indicated that after 30 days, residues for pydiflumetofen, benzovindiflupyr, prothioconazole-desthio and mefentrifluconazole were 33.8, 18.4, 33.3 and 50.7% of their initial detected concentration, respectively. Most fungicide degradation followed first-order reaction kinetics, where the half-life for each fungicide could be determined. Prothioconazole-desthio was observed as having the longest half-life. The combination of prothioconazole-desthio being able to inhibit *S. vaccinii* growth and its long persistence time, could be reasons for its ability to effectively management foliar diseases in the field. The field trial results reinforce this speculation.

By analysing fungicide sensitivity and persistence, the two can be discussed in the context of the protection provided at certain time in points after application. For example, the average benzovindiflupyr EC_{50} concentration for *S. vaccinii* and *T. minima*, was 10.1 and 0.005 $\mu g \cdot mL^{-1}$, respectively. In comparison, 30 days after application,

benzovindiflupyr was observed in the leaf tissue at 1.67 $mg \cdot kg^{-1}$. The units here can be viewed as both being parts per million. Based on these results, benzovindiflupyr would provide minimal effect on *S. vaccinii* infection, but still maintain protection from *T. minima*, 30 days after application. Additionally, the average prothioconazole-desthio EC50 concentration for *S. vaccinii* and *T. minima*, was 0.82 and 5.4 $\mu g \cdot mL^{-1}$, respectively. As for the residue concentration, 30 days after application prothioconazole-desthio was observed in the leaf tissue at 0.78 $mg \cdot kg^{-1}$. Based on this, prothioconazole-desthio could still be inhibiting *S. vaccinii* growth, however is likely providing minimal inhibition to *T. minima*. The link between EC_{50} and persistence concentrations provides insight into how and why these fungicides provide protection from pathogens in the field.

5.1 Overall Conclusions

In general, this study provides detailed insight into the efficacy, sensitivity and persistence of fungicides used on wild blueberries for managing foliar diseases. This study focussed on key knowledge gaps that were outlined by the industry. The results from this study will be very useful for the wild blueberry industry to guide disease management decisions that extend far beyond just the efficacy of fungicides in the field. The results will help inform decisions and answer questions about resistance issues with current fungicides and ones that will be used decades from now. In addition, a common question surrounding foliar disease management is whether one application is sufficient. The results of the persistence portion of the study provided evidence that fungicides do remain present in the leaves for over 30 days, however they may be at concentrations that fall below those that would inhibit fungal growth.

While this study contains quantitative evidence surrounding fungicide use, areas of future research are noted. Disease management should be thought of in an integrated approach with also encompasses cultural and biological management methods. Previous studies on wild blueberries have found that Botrytis blight can be reduced by the use of lime sulphur treatments combined with thermal treatment or a biofungicide during the plant's dormancy. Similar studies should be conducted to evaluate these pest control methods for their ability to reduce the primary inoculum associated with Sphaerulina leaf spot and leaf rust. Another area that is gaining interest around the world in biological control methods. Biological control methods have been studied on wild blueberries for managing floral diseases, however; minimal research has been done on their use for managing foliar diseases. With that said, few biological control methods that are specific to leaf diseases have been presented to the industry or trialed on wild blueberries. As consumers of fruit and vegetables show greater interest in residue free produce or regenerative agriculture, greater interest in biological controls methods may be generated and result in more research in this topic.

REFERENCES

Abbasi, P. A., Hildebrand, P. D., Ali, S., Moreau, D. L., & Renderos, W. E. (2022). Effect of RH, temperature, light, and plant age on infection of lowbush blueberry by *Sphaerulina vaccinii*. *Plant Disease, 106*(1), 297-303. https://doi.org/10.1094/PDIS-12-20-2747-RE

Abbey, J. (2017). *Sustainable management of botrytis blossom blight in wild blueberry (Vaccinium angustifolium Aiton)*. [Master's thesis, Dalhousie University]. Novanet.

Abbey, J. (2022). *Wild blueberry (Vaccinium spp.) – Microbe interactions and botrytis blight management.* [Master's thesis, Dalhousie University]. Novanet.

Abbey, J. A., Percival, D., Anku, K., Schilder, A., & Asiedu, S. K. (2022). Managing botrytis blossom blight of wild blueberry through field sanitation, lime sulfur and *Trichoderma* application. *Canadian Journal of Plant Pathology, 44*(3), 361-371. https://doi.org/10.1080/07060661.2021.2023649

Agriculture and Agri-Food Canada. (2022). Statistical overview of the Canadian fruit industry. https://agriculture.canada.ca/en/sector/horticulture/reports/statistical-overview-canadian-fruit-industry-2021#a1.2

Agrios, G. N. (2005). *Plant pathology (5th Edition)*. Elsevier Academic Press.

Ali, S., Hildebrand, P. D., Renderos, W. E., & Abbasi, P. A. (2021). Identification and characterization of *Sphaerulina vaccinii* as the cause of leaf spot and stem canker in lowbush blueberry and its epidemiology. *Phytopathology, 111*(9), 1560-1570. https://doi.org/10.1094/phyto-04-20-0143-r

Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International, 86*(2), 412–431. https://doi.org/10.1093/jaoac/86.2.412

Anderson, S. C., Christiansen, A., Peterson, A., Beukelman, L., & Nienow, A. M. (2016). Statistical analysis of the photodegradation of imazethapyr on the surface of extracted soybean (*Glycine max*) and corn (*Zea mays*) epicuticular waxes. *Environmental Science: Processes & Impacts, 18*(10), 1305–1315. https://doi.org/10.1039/C6EM00401F

Angioni, A., Aguilera Del Real, A., Russo, M., Melis, M., Cabitza, F., & Cabras, P. (2003). Triazole fungicide degradation in peaches in the field and in model systems. *Food Additives and Contaminants, 20*(4), 368–374. https://doi.org/10.1080/0265203021000060904

Angioni, Alberto, Cabizza, M., Cabras, M., Melis, M., Tuberoso, C., & Cabras, P. (2004). Effect of the epicuticular waxes of fruits and vegetables on the photodegradation of rotenone. *Journal of Agricultural and Food Chemistry, 52*(11), 3451–3455. https://doi.org/10.1021/JF035344Y/ASSET/IMAGES/LARGE/JF035344YF00005.JPEG

Anku, K., Percival, D., Rajasekaran, L., Heung, B., & Vankoughnett, M. (2023). Phenological assessment of the wild blueberry field using an unmanned aerial vehicle. *Acta Horticulturae,* 35-42. https://doi.org/10.17660/ActaHortic.2023.1357.6

Arena, M., Auteri, D., Barmaz, S., Bellisai, G., Brancato, A., Brocca, D., … Villamar-Bouza, L. (2018). Peer review of the pesticide risk assessment of the active substance chlorothalonil. *EFSA Journal*. https://doi.org/10.2903/j.efsa.2018.5126

Arneson, P., Oren, T., Loria, R., Jenkins, J., Goodman, E., & Cooper, W. (2002). Management of apple scab; Simulation with Applescab. Cornell University. http://www.scientificsocieties.org/aps/AppleScab/Applescab_Manual.html

Assmann, J. J., Kerby, J. T., Cunliffe, A. M., & Myers-Smith, I. H. (2019). Vegetation monitoring using multispectral sensors — best practices and lessons learned from high latitudes. *Journal of Unmanned Vehicle Systems*. https://doi.org/10.1139/juvs-2018-0018

Augusto, J., & Brenneman, T. B. (2012). Assessing systemicity of peanut fungicides through bioassay of plant tissues with *Sclerotium rolfsii*. *Plant Disease, 96*(3), 330-337. https://doi.org/10.1094/PDIS-04-11-0303

Bakirci, G. T., & Hişil, Y. (2012). Fast and simple extraction of pesticide residues in selected fruits and vegetables using tetrafluoroethane and toluene followed by ultrahigh-performance liquid chromatography/tandem mass spectrometry. *Food Chemistry, 3*(1), 1901-1913. https://doi.org/10.1016/j.foodchem.2012.06.051

Bernardi, G., Kemmerich, M., Ribeiro, L. C., Adaime, M. B., Zanella, R., & Prestes, O. D. (2016). An effective method for pesticide residues determination in tobacco by GC-MS/MS and UHPLC-MS/MS employing acetonitrile extraction with low-temperature precipitation and d-SPE clean-up. *Talanta, 161*, 40–47. https://doi.org/10.1016/J.TALANTA.2016.08.015

Birr, T., Hasler, M., Verreet, J. A., & Klink, H. (2021). Temporal changes in sensitivity of *Zymoseptoria tritici* field populations to different fungicidal modes of action. *Agriculture (Switzerland)*, *11*(269). https://doi.org/10.3390/agriculture11030269

Bleyer, G., Lösch, F., Schumacher, S., & Fuchs, R. (2020). Together for the better: Improvement of a model based strategy for grapevine downy mildew control by addition of potassium phosphonates. *Plants, 9*(6). https://doi.org/10.3390/plants9060710

Brent, K. J., & Hollomon, D. W. (2007). *Fungicide resistance in crop pathogens: How can it be managed? FRAC Monograph 1, 2nd edition, Brussels: FRAC*.

Breunig, M., & Chilvers, M. I. (2021). Baseline sensitivity of *Fusarium graminearum* from wheat, corn, dry bean and soybean to pydiflumetofen in Michigan, USA. *Crop Protection*. https://doi.org/10.1016/j.cropro.2020.105419

Breunig, M., & Chilvers, M. (2022). Comparison between prothioconazole and prothioconazole-desthio in poison-plate mycelial growth assays of *Fusarium graminearum*. *Plant Heath Progress, 23*(2), 159-161. https://doi.org/10.1094/PHP-06-21-0087-RS

Bromilow, R. H., Evans, A. A., & Nicholls, P. H. (1999). Factors affecting degradation rates of five triazole fungicides in two soil types: 1 Laboratory incubations. *Pesticide Science, 55*(12), 1129–1134. https://doi.org/10.1002/ps.2780551202

Buck, J. W., & Williams-Woodward, J. L. (2003). The effect of fungicides on urediniospore germination and disease development of daylily rust. *Crop Protection, 22*(1), 135-140. https://doi.org/10.1016/S0261-2194(02)00123-0

Burlakoti R., & Percival D. (2023). Sensitivity of *Septoria* spp. associated with lowbush blueberry foliar disease to chlorothalonil and propiconazole fungicide. *Acta Hortic,* 251-256. https://doi.org/10.17660/ActaHortic.2023.1357.36

Cabras, P., Angioni, A., Garau, V. L., Melis, M., Pirisi, F. M., Cabitza, F., & Pala, M. (2001). The effect of simulated rain on folpet and mancozeb residues on grapes and on vine leaves. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 36*(5), 609-618. https://doi.org/10.1081/PFC-100106189

Caffi, T., & Rossi, V. (2018). Fungicide models are key components of multiple modelling approaches for decision-making in crop protection. *Phytopathologia Mediterranea, 57*(1), 153-169. https://doi.org/10.14601/Phytopathol_Mediterr-22471

Carmona, M., Sautua, F., Pérez-Hérnandez, O., & Reis, E. M. (2020). Role of fungicide applications on the integrated management of wheat stripe rust. *Frontiers in Plant Science, 11*(733). https://doi.org/10.3389/fpls.2020.00733

Castillo, S., Borrero, C., Castaño, R., Rodríguez, A., & Avilés, M. (2013). First report of canker disease caused by *Neofusicoccum parvum* and *N. australe* on blueberry bushes in Spain. *Plant Disease, 97*(8) https://doi.org/10.1094/PDIS-11-12-1048-PDN

Chai, L. K., Mohd-Tahir, N., & Hansen, H. C. B. (2009). Dissipation of acephate, chlorpyrifos, cypermethrin and their metabolites in a humid-tropical vegetable production system. *Pest Management Science, 65*(2), 189–196. https://doi.org/10.1002/PS.1667

Chen, H., Yin, P., Wang, Q., Jiang, Y., & Liu, X. (2014). A modified QuEChERS sample preparation method for the analysis of 70 pesticide residues in tea using gas chromatography-tandem mass spectrometry. *Food Analytical Methods, 7*(8), 1577–1587. https://doi.org/10.1007/S12161-014-9791-0/TABLES/2

Chollet, J. F., Rocher, F., Jousse, C., Delétage-Grandon, C., Bashiardes, G., & Bonnemain, J. L. (2004). Synthesis and phloem mobility of acidic derivatives of the fungicide fenpiclonil. *Pest Management Science, 60*(11), 1063-1072. https://doi.org/10.1002/ps.906

Conti, M., Cinget, B., Vivancos, J., Oudemans, P., & Belanger, R. (2019). A molecular assay allows the simultaneous detection of 12 fungi causing fruit rot in cranberry. *Plant Disease, 103*(11), 2843-2850. https://doi.org/10.1094/PDIS-03-19-0531-RE

Crowe, K. M., Bushway, A. A., Bushway, R. J., & Davis-Dentici, K. (2007). Microbial degradation of phosmet on blueberry fruit and in aqueous systems by indigenous bacterial flora on lowbush blueberries (*Vaccinium angustifolium*). *Journal of Food Science, 72*(8), 293–299. https://doi.org/10.1111/J.1750-3841.2007.00466.X

D'Appollonio, J. (2018a). *Red sorrel*. The University of Maine Extension. https://extension.umaine.edu/blueberries/weed-images/herbaceous-broadleaf-weeds/redorange-flowers/red-sorrel/

D'Appollonio, J. (2018b). *Fineleaf sheep fescue*. The University of Maine Extension. https://extension.umaine.edu/blueberries/weed-images/grasses-sedges-rushes/fineleaf-sheep-fescue/

Davies, L. O., Bramke, I., France, E., Marshall, S., Oliver, R., Nichols, C., … Bending, G. D. (2013). Non-UV light influences the degradation rate of crop protection products. *Environmental Science and Technology, 47*(15), 8229–8237. https://doi.org/10.1021/ES402139X

Dawson, J. K. (2009). *Impact of foliar diseases on carbohydrate dynamics of the wild blueberry (Vaccinium angustifolium Ait.)* (ISBN: 978-0-494-56350-2) [Master's thesis, Nova Scotia Agricultural College]. Novanet.

Dec, J., Haider, K., Benesi, A., Rangaswamy, V., Schäffer, A., Plücken, U., & Bollag, J. M. (1997). Analysis of soil-bound residues of 13C-labeled fungicide cyprodinil by NMR spectroscopy. *Environmental Science and Technology, 31*(4), 1128-1135. https://doi.org/10.1021/es960631m

Deising, H. B., Reimann, S., & Pascholati, S. F. (2008). Mechanisms and significance of fungicide resistance. *Brazilian Journal of Microbiology, 39*(2), 286-295. https://doi.org/10.1590/S1517-83822008000200017

Ding, S., Halterman, D. A., Meinholz, K., & Gevens, A. J. (2019). Distribution and stability of quinone outside inhibitor fungicide resistance in populations of potato pathogenic *Alternaria* spp. In Wisconsin. *Plant Disease, 103*, 2033-2040. https://doi.org/10.1094/PDIS-11-18-1978-RE

Dong, X., Tong, Z., Chu, Y., Sun, M., Wang, M., Gao, T., & Duan, J. (2019). Dissipation of prothioconazole and its metabolite prothioconazole-desthio in rice fields and risk assessment of its dietary intake. *Journal of Agricultural and Food Chemistry, 67*(23), 6458-6465. https://doi.org/10.1021/acs.jafc.8b06788

Edgington, L. V. (1981). Structural Requirements of Systemic Fungicides. *Annual Review of Phytopathology*. https://doi.org/10.1146/annurev.py.19.090181.000543

European Food Safety Authority. (2015). Conclusion on the peer review of the pesticide risk assessment of the active substance benzovindiflupyr. *EFSA Journal,13*(3). doi:10.2903/j.efsa.2015.4043

European Food Safety Authority. (2021). Modification of the existing maximum residue levels for benzovindiflupyr in fresh herbs and edibleflowers. *EFSA Journal, 19*(9). doi: 10.2903/j.efsa.2021.6839

Farooque, A. A., Zaman, Q. U., Schumann, A. W., Madani, A., & Percival, D. C. (2012). Response of wild blueberry yield to spatial variability of soil properties. *Soil Science, 177*(1), 56-68. https://doi.org/10.1097/SS.0b013e3182376ed6

Food and Agriculture Organization. (2016). Joint FAO/WHO Meeting on Pesticide Residues: Benzovindiflupy /https://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report2016/BENZOVINDIFLUPYR.pdf

Garnica, D. P., Upadhyaya, N. M., Dodds, P. N., & Rathjen, J. P. (2013). Strategies for wheat stripe rust pathogenicity identified by transcriptome sequencing. *PLoS ONE, 8*(6). https://doi.org/10.1371/journal.pone.0067150

Glass, V. (2000). *Drought stress effects in wild blueberries* (*Vaccinium angustifolium* Ait). Master's Thesis. Dalhousie University, Halifax, Nova Scotia. (ISBN: 0612572374).

Golembiewski, R. C., Vargas, J. M., Jones, A. L., & Detweiler, A. R. (1995). Detection of demethylation inhibitor (DMI) resistance in *Sclerotinia homoeocarpa* populations. *Plant Disease, 79*, 491-493. https://doi.org/10.1094/PD-79-0491

Government of New Brunswick Agriculture, Aquaculture and Fisheries. (2010). *Growth and development of the wild blueberry.* Wild Blueberry Fact Sheet A.2.0. https://www2.gnb.ca/content/dam/gnb/Departments/10/pdf/Agriculture/WildBlueberries-BleuetsSauvages/a20e.pdf

Grimalt, S., & Dehouck, P. (2016). Review of analytical methods for the determination of pesticide residues in grapes. *Journal of Chromatography A, 1433*, 1-23 https://doi.org/10.1016/j.chroma.2015.12.076

Hall, I. V., Forsyth, F. R., Aalders, L. E., & Jackson, L. P. (1972). Physiology of the lowbush blueberry. *Economic Botany, 26*(1), 68-73. https://doi.org/10.1007/BF02862263

Hamed, S. M., Al-Nuaemi, I. J., Korany, S. M., Alsherif, E. A., Mohamed, H. S., & Abdelgawad, H. (2022). Hazard assessment and environmental fate of propiconazole degradation by microalgae: Differential tolerance, antioxidant and detoxification pathway. *Journal of Environmental Chemical Engineering, 10*(4), 108170. https://doi.org/10.1016/J.JECE.2022.108170

Hergueta-Castillo, M., Lopez-Ruiz, R., Frenich, A., & Romero-Gonzalez, R. (2023). Understanding the metabolism and dissipation kinetics of flutriafol in vegetables under laboratory and greenhouse scenarios. *Foods, 12*(1). https://doi.org/10.3390/foods12010201

Heshmati, A., Komacki, H. A., Nazemi, F., & Mousavi Khaneghah, A. (2020). Persistence and dissipation behavior of pesticide residues in parsley (*Petroselinum crispum*) under field conditions. *Quality Assurance and Safety of Crops & Foods, 12*(3), 55–65. https://doi.org/10.15586/QAS.V12I3.755

Heshmati, A., Mehri, F., & Mousavi Khaneghah, A. (2021). Simultaneous multi-determination of pesticide residues in black tea leaves and infusion: a risk assessment study. *Environmental Science and Pollution Research, 28*(11), 13725-13735. https://doi.org/10.1007/s11356-020-11658-3

Hildebrand, P.D., Renderos, W.E., & R.W. Delbridge. (2016). Diseases of lowbush blueberry and their identification. *Agriculture and Agri-Food Canada*. (No. 12476E).

Hildebrand, P., Renderos, W., Filmore, S., & Walker, B. (2010). Symptoms and epidemiology of a *Septoria* spp. causing leaf and stem canker of lowbush blueberry. *Canadian Journal of Plant Pathology, 32*(3).

Hildebrand, P., Renderos, W., & Burgess, P. (2011). *Wild Blueberry Fact Sheet: Septoria Leaf Spot and Stem Canker*. Wild Blueberry Producers Association of Nova Scotia.

Hsiang, T., Yang, L., & Barton, W. (1997). Baseline sensitivity and cross-resistance to demethylation-inhibiting fungicides in Ontario isolates of *Sclerotinia homoeocarpa*. *European Journal of Plant Pathology, 103*, 409-416. https://doi.org/10.1023/A:1008671321231

Hopkins, L., Norton, L., & Gyrisco, G. (1952). Persistence of insecticide residues on forage crops. *Journal of Economic Entomology, 45*(2), 213-218. https://doi.org/10.1093/jee/45.2.213

Huarhua, M., Acuña, R., Aragón, L., Soto, J., Landeo, S., de la Parte, E. M., & Apaza, W. (2020). First report of blueberry leaf rust caused by *Thekopsora minima* on *Vaccinium corymbosum* in Peru. *Plant Disease*, *104* (11). https://doi.org/10.1094/PDIS-03-20-0585-PDN

Ishii, H., Bryson, P. K., Kayamori, M., Miyamoto, T., Yamaoka, Y., & Schnabel, G. (2021). Cross-resistance to the new fungicide mefentrifluconazole in DMI-resistant fungal pathogens. *Pesticide Biochemistry and Physiology*. https://doi.org/10.1016/j.pestbp.2020.104737

Juliatti, F., L., Polloni, T., de Moraid, N., Zacarias, E., Silva, & B., Juliatti. (2017). Sensitivity of *Phakopsora pachyrhizi* populations to dithiocarbamate, chloronitrile, triazole, strobilurin and carboxamide fungicides. *Bioscience Journal¸ 33*(4):933-943. DOI: 10.14393/BJ-v33n4a2017-38357

Juliatti, F., & L., Zambolim. (2021). Etiology, epidemiology and management of Asian soybean rust (ASR) in Brazil and vulnerability of chemical control of specific without multisite fungicides. In A. K. Goyal (Eds.), *Cereal Grains; Volume 1*. IntechOpen. DOI: 10.5772/intechopen.97686

Jacobsen, R. E., Fantke, P., & Trapp, S. (2015). Analysing half-lives for pesticide dissipation in plants. *SAR and QSAR in Environmental Research, 26*(4), 325–342. https://doi.org/10.1080/1062936X.2015.1034772

Kang, Z., Li, X., Wan, A., Wang, M., & Chen, X. (2019). Differential sensitivity among *Puccinia striiformis* f. sp. *tritici* isolates to propiconazole and pyraclostrobin fungicides. *Canadian Journal of Plant Pathology*, *41*(3), 415-434. https://doi.org/10.1080/07060661.2019.1577301

Karns, J. S., Mulbry, W. W., Nelson, J. O., & Kearney, P. C. (1986). Metabolism of carbofuran by a pure bacterial culture. *Pesticide Biochemistry and Physiology, 25*(2), 211–217. https://doi.org/10.1016/0048-3575(86)90048-9

Katagi, T. (2004). Photodegradation of pesticides on plant and soil surfaces. *Reviews of Environmental Contamination and Toxicology, 182*, 1–195. https://doi.org/10.1007/978-1-4419-9098-3_1

Kemen, A. C., Agler, M. T., & Kemen, E. (2015). Host-microbe and microbe-microbe interactions in the evolution of obligate plant parasitism. *New Phytologist, 206*(4), 1207-1228. https://doi.org/10.1111/nph.13284

Kiiker, R., Juurik, M., Heick, T. M., & Mäe, A. (2021). Changes in DMI, SDHI, QOI fungicide sensitivity in the Estonian *Zymoseptoria tritici* population between 2019 and 2020. *Microorganisms, 9*(4). https://doi.org/10.3390/microorganisms9040814

Kinsman, G. (1986). *The history of the lowbush blueberry industry in Nova Scotia, 1880-1950*. The Blueberry Producers' Association of Nova Scotia. https://dalspace.library.dal.ca//handle/10222/27987

Klittich, C. J. R., & Ray, S. L. (2013). Effects of physical properties on the translaminar activity of fungicides. *Pesticide Biochemistry and Physiology, 107*(3), 351-359. https://doi.org/10.1016/j.pestbp.2013.10.003

Koller, W., & H., Scheinpflug. (1987). Fungal resistance to sterol biosvnthesis inhibitors: A new challenge. *The American Phytopathological Society, 71*, 1066-1107. DOI: 10.1094/PD-71-1066.

Kuznetsov, D., Cazenave, A. B., Rambach, O., Camblin, P., Nina, M., & Leipner, J. (2018). Foliar application of benzovindiflupyr shows non-fungicidal effects in wheat plants. *Pest Management Science*, *74*(3), 665-671. https://doi.org/10.1002/ps.4754

Landschoot, P. (2021). *Turfgrass diseases: leaf spot and melting-out diseases (causal fungi: Bipolaris and Drechslera spp.)*. PennState Extension. https://extension.psu.edu/turfgrass-diseases-leaf-spot-and-melting-out-diseases-causal-fungi-bipolaris-and-drechslera-spp

Lehoczki-Krsjak, S., Varga, M., Szabó-Hevér, Á., & Mesterházy, Á. (2013). Translocation and degradation of tebuconazole and prothioconazole in wheat following fungicide treatment at flowering. *Pest Management Science, 69*(11), 1216-1224, https://doi.org/10.1002/ps.3486

Lesemann, S. S., Schimpke, S., Dunemann, F., & Deising, H. B. (2006). Mitochondrial heteroplasmy for the cytochrome b gene controls the level of strobilurin resistance in the apple powdery mildew fungus *Podosphaera leucotricha* (Ell. & Ev.) E.S. Salmon. *Journal of Plant Diseases and Protection, 113*, 259-266. https://doi.org/10.1007/BF03356191

Li, Y. H., Trigiano, R. N., Windham, M. T., Vito, L. M., Fare, D. C., Spiers, J. M., & Copes, W. E. (2009). Inhibition of urediniospore germination in *Puccinia hemerocallidis* by Bacto Agar and changes in percent germination and germ-tube elongation on agarose over time. *Canadian Journal of Plant Pathology, 31*(2), 163-168. https://doi.org/10.1080/07060660909507589

Li, L., Sun, X., Zhao, X., Xiong, Y., Gao, B., Zhang, J., … Wang, M. (2021). Absolute configuration, enantioselective bioactivity, and degradation of the novel chiral triazole fungicide mefentrifluconazole. *Journal of Agricultural and Food Chemistry, 69*(17), 4960–4967. https://doi.org/10.1021/ACS.JAFC.0C07947/ASSET/IMAGES/LARGE/JF0C07947_0005.JPEG

Lin, H. F., Dong, B., & Hu, J. Y. (2017). Residue and intake risk assessment of prothioconazole and its metabolite prothioconazole-desthio in wheat field. *Environmental Monitoring and Assessment, 189*(5). https://doi.org/10.1007/s10661-017-5943-1

Lloyd, A., Percival, D., & Yurgel, S. (2021). Effect of fungicide application on lowbush blueberry soil microbiome. *Microorganisms, 9*(7). https://doi.org/10.3390/microorganisms9071366

Lobos, G. A., & Hancock, J. F. (2015). Breeding blueberries for a changing global environment: A review. *Frontiers in Plant Science, 6*(782). https://doi.org/10.3389/fpls.2015.00782

Loladze, A., Rodrigues, F. A., Toledo, F., Vicente, F. S., Gérard, B., & Boddupalli, M. P. (2019). Application of remote sensing for phenotyping tar spot complex resistance in maize. *Frontiers in Plant Science, 10*(552). https://doi.org/10.3389/fpls.2019.00552

MacDonald, J., & Lyu, H. (2022). *Nova Scotia Guide to Pest Management in Wild Blueberry 2022*. Perennia Food and Agriculture Corporation.

Maqbool, R., Percival, D., Zaman, Q., Astatkie, T., Adl, S., & Buszard, D. (2016). Improved growth and harvestable yield through optimization of fertilizer rates of soil-applied nitrogen, phosphorus, and potassium in wild blueberry (*Vaccinium angustifolium* ait.). *HortScience, 51*(9), 1092-1097. https://doi.org/10.21273/HORTSCI08204-16

Martinez-Espinoza, A., Sawyer, A., Youmans, J., & Buck, J. (2014). *Identification and control of leaf rust of wheat of leaf rust of wheat in Georgia*. University of Georgia Extension. https://extension.uga.edu/publications/detail.html?number=C1060&title=identification-and-control-of-leaf-rust-of-wheat-in-georgia

Miles, J., Sans, W., Wressell, H., & Manson, G. (1963). Growth-dilution as a factor in the decline of pesticide residues on alfalfa-grass forage. *Canadian Journal of Plant Science, 44*, 37-41.

Mondal, S. N., Vicent, A., Reis, R. F., & Timmer, L. W. (2007). Efficacy of pre- and postinoculation application of fungicides to expanding young citrus leaves for control of melanose, scab, and alternaria brown spot. *Plant Disease, 91*(12), 1600-1606. https://doi.org/10.1094/PDIS-91-12-1600

Müller, M. A., Stammler, G., & May De Mio, L. L. (2021). Multiple resistance to DMI, QoI and SDHI fungicides in field isolates of *Phakopsora pachyrhizi. Crop Protection, 145.* https://doi.org/10.1016/j.cropro.2021.105618

Nguyen, N. (2019). *The dispersal pattern of Thekopsora minima in wild blueberry determined by a molecular detection method* [Master's Thesis, University of Maine]. Digital Commons.

Oellig, C. (2016). Acetonitrile extraction and dual-layer solid phase extraction clean-up for pesticide residue analysis in propolis. *Journal of Chromatography A*, 1445, 19–26. https://doi.org/10.1016/J.CHROMA.2016.03.082

Ojiambo, P. S., Scherm, H., & Brannen, P. M. (2006). Septoria leaf spot reduces flower bud set and yield potential of rabbiteye and southern highbush blueberries. *Plant Disease, 90*, 51-57. https://doi.org/10.1094/PD-90-0051

Olson, D., & Anderson, J. (2021). Review on unmanned aerial vehicles, remote sensors, imagery processing, and their applications in agriculture. *Agronomy Journal, 113*(2), 971-992. https://doi.org/10.1002/agj2.20595

Parker, J. E., Warrilow, A. G. S., Cools, H. J., Martel, C. M., David Nes, W., Fraaije, B. A., … Kelly, S. L. (2011). Mechanism of binding of prothioconazole to *Mycosphaerella graminicola* CYP51 differs from that of other azole antifungals. *Applied and Environmental Microbiology, 77*(4), 1460-1465. https://doi.org/10.1128/AEM.01332-10

Paul, P. A., Lipps, P. E., Hershman, D. E., McMullen, M. P., Draper, M. A., & Madden, L. V. (2008). Efficacy of triazole-based fungicides for Fusarium head blight and deoxynivalenol control in wheat: A multivariate meta-analysis. *Phytopathology, 98*(9), 999-1011. https://doi.org/10.1094/PHYTO-98-9-0999

Percival, D. C., Abbey, J., Lu, H., & Harris, L. (2017). Use of biofungicides to address conventional botrytis blight control challenges in wild blueberry production. *Proceedings from the XI International Vaccinium Symposium*. https://doi.org/10.17660/ActaHortic.2017.1180.32

Percival, D., & Burnham, J. (2009). Impact of mycorrhizal association and soil-applied nitrogen and phosphorous on lowbush blueberry (*Vaccinium angustifolium* Ait.). *International Society for Horticultural Science. Annals of Botany*. https://doi.org/10.1093/aob/mcn241

Percival, D. C., & Dawson, J. K. (2009). Foliar disease impact and possible control strategies in wild blueberry production. *Proceedings from the IX International Vaccinium Symposium*. https://doi.org/10.17660/ActaHortic.2009.810.45

Pescie, M., Lovisolo, M., De Magistris, A., Strik, B., & López, C. (2011). Flower bud initiation in southern highbush blueberry cv. O'Neal occurs twice per year in temperate to warm temperate conditions. *Journal of Applied Horticulture, 13*(1), 8-12. https://doi.org/10.37855/jah.2011.v13i01.02

Pest Management Regulatory Agency. (2022). *Proposed special review decision PSRD2022-01 Special review of chlorothalonil and its associated end-use products* (ISSN: 2561-636). https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/consultations/proposed-special-review-decision/2022/chlorothalonil/document.html

Pethybridge, S. J., Sharma, S., Hansen, Z. A. C. H. A. R. I. A. H. R., Vaghefi, N., Hanson, L. E., & Kikkert, J. R. (2020). Improving fungicide-based management of Cercospora leaf spot in table beet in New York, USA. *Canadian Journal of Plant Pathology*, *42*(3), 353-366. https://doi.org/10.1080/07060661.2019.1690048

Pfister, S. E., Halik, S., & Bergdahl, D. R. (2004). Effect of temperature on *Thekopsora minima* urediniospores and uredinia. *Plant Disease, 88*(4), 359-362. https://doi.org/10.1094/PDIS.2004.88.4.359

Polashock, J., Caruso, F., Averill, A., & Schilder, C. (2017). Compendium of Blueberry, Cranberry and Lingonberry Diseases and Pests (2nd Edition). American Phytopathological Society.

Pontzen, R., & Scheinpflug, H. (1989). Effects of triazole fungicides on sterol biosynthesis during spore germination of *Botrytis cinerea*, *Venturia inaequalis* and *Puccinia graminis* f. sp. *tritici*. *Netherlands Journal of Plant Pathology, 95*, 151-160. https://doi.org/10.1007/BF01974294

Privé, J. P., Russell, L., Hildebrand, P., Boyd, N., & Percival, D. C. (2012). Intelligent irrigation of lowbush blueberries in Atlantic Canada. *Acta Horticulturae, 962*, 471-476. https://doi.org/10.17660/ActaHortic.2012.926.66

Quaedvlieg, W., Verkley, G. J. M., Shin, H. -D., Barreto, R. W., Alfenas, A. C., Swart, W. J., Groenewald, J. Z., & Crous, P. W. 2013. Sizing up *Septoria*. *Studies in Mycology 75*, 307–390. https://doi.org/10.3114/sim0017

Rampersad, S. N. (2011). A rapid colorimetric microtiter bioassay to evaluate fungicide sensitivity among *Verticillium dahliae* isolates. *Plant Disease, 95*(3), 248-255. https://doi.org/10.1094/PDIS-10-10-0725

Retamales, J., & Hancock, J. (2012). *Blueberries*. Cabi: Cambridge, MA

Roloff, I., Scherm, H., & van Iersel, M. W. (2004). Photosynthesis of blueberry leaves as affected by septoria leaf spot and abiotic leaf damage. *Plant Disease, 88*(4), 397-401. https://doi.org/10.1094/PDIS.2004.88.4.397

Romero, J., Ávila, A., Agustí-Brisach, C., Roca, L. F., & Trapero, A. (2020). Evaluation of fungicides and management strategies against cercospora leaf spot of olive caused by Pseudocercospora cladosporioides. *Agronomy, 10*(2), 271. https://doi.org/10.3390/agronomy10020271

Ryckaert, B., Spanoghe, P., Haesaert, G., Heremans, B., Isebaert, S., & Steurbaut, W. (2007). Quantitative determination of the influence of adjuvants on foliar fungicide residues. *Crop Protection, 26*(10), 1589-1594. https://doi.org/10.1016/j.cropro.2007.02.011

Russell, P. (2002). *Sensitivity Baseline in Fungicide Resistance Research and Management.* Crop Life International.

Sang, H., Popko, J. T., & Jung, G. (2019). Evaluation of a *Sclerotinia homoeocarpa* population with multiple fungicide resistance phenotypes under differing selection pressures. *Plant Disease, 103*(4), 685-690. https://doi.org/10.1094/PDIS-06-18-1080-RE

SANCO/12745/2013 (2013). Validation of the MRM pesticides from the working document SANCO/12745/2013 of 21-22 November 2017 rev. 9(1). https://www.eurl-pesticides.eu/userfiles/file/Report_Validation_WorkinDocument_2018_DRAFT.pdf

SANTE/12682/2019 (2020). *Main changes introduced in document No SANTE/12682/2019 with respect to the previous* version (Document No SANTE/11813/2017) *Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed*. https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727

Sarraf, M., Beig-babaei, A., & Naji-Tabasi, S. (2020). Application of QuEChERS method for extraction of functional compounds. *SN Applied Sciences, 2*(11), 1–6. https://doi.org/10.1007/S42452-020-03639-5/FIGURES/4

Satchivi, N. M. (2014). Modeling xenobiotic uptake and movement: A review. In *ACS Symposium Series.* https://doi.org/10.1021/bk-2014-1171.ch003

Schilder, A. (2010). *Fungicide properties and weather conditions.* Michigan State University Extension. https://www.canr.msu.edu/news/fungicide_properties_and_weather_conditions

Shands, A. C., Crandall, S. G., Ho, T., & Miles, T. D. (2018). First report of leaf rust on southern highbush blueberry caused by *Thekopsora minima* in California. *Plant Disease, 102*(6). https://doi.org/10.1094/PDIS-09-17-1439-PDN

Siegel, M. R. (1981). Sterol-inhibiting fungicides: Effects on sterol biosynthesis and sites of action. *Plant Disease*, *65*, 986-989 https://doi.org/10.1094/pd-65-986

Silva, É. A. S., Lopez-Avila, V., & Pawliszyn, J. (2013). Fast and robust direct immersion solid phase microextraction coupled with gas chromatography-time-of-flight mass spectrometry method employing a matrix compatible fiber for determination of triazole fungicides in fruits. *Journal of Chromatography A, 1313*, 139-146. https://doi.org/10.1016/j.chroma.2013.07.071

Simón, M. R., Fleitas, M. C., Castro, A. C., & Schierenbeck, M. (2020). How foliar fungal diseases affect nitrogen dynamics, milling, and end-use quality of wheat. *Frontiers in Plant Science, 11:569401*. https://doi.org/10.3389/fpls.2020.569401

Simpson, M., Wilk, P., Collins, D., Robertson, D., & Daniel, R. (2017). Managing blueberry rust under an evergreen system. *Proceedings from the XI International Vaccinium Symposium*. https://doi.org/10.17660/ActaHortic.2017.1180.14

Skrzydeł, J., Borowska-wykręt, D., & Kwiatkowska, D. (2021). Structure, assembly and function of cuticle from mechanical perspective with special focus on Perianth. *International Journal of Molecular Sciences, 22*(8), 22. https://doi.org/10.3390/IJMS22084160

Slawecki, R. A., Ryan, E. P., & Young, D. H. (2002). Novel fungitoxicity assays for inhibition of germination-associated adhesion of *Botrytis cinerea* and *Puccinia recondita* spores. *Applied and Environmental Microbiology, 68*(2), 597-601. https://doi.org/10.1128/AEM.68.2.597-601.2002

Smagula, J., & DeGomez, T. (1987). *Leaf and soil sampling procedures*. University of Maine, Wild Blueberry Factsheet No. 222. https://extension.umaine.edu/blueberries/factsheets/production/leaf-soil-sampling-procedures/

Song, G., & Hancock, J. (2011). *Vaccinium*. In C. Kole (Eds.), *Wild Crop Relatives: Genomic and Breeding Resources, Temperate Fruits*. Springer. http://dx.doi.org/10.1007/978-3-642-16057-8_10

Strushkevich, N., Usanov, S. A., & Park, H. W. (2010). Structural basis of human CYP51 inhibition by antifungal azoles. *Journal of Molecular Biology, 97*(4)1067-78. https://doi.org/10.1016/j.jmb.2010.01.075

Sun, X., Yan, X., Yang, B., Ding, Y., & Yu, X. (2018). Degradation dynamics and residue analysis of prothioconazole and its metabolite prothioconazole-desthio in wheat and soil by QuEChERS-high performance liquid chromatography-tandem mass spectrometry. *Food Science, 39*(22), 269-275. https://doi.org/10.7506/spkx1002-6630-201822041

Talley, S. M., Coley, P. D., & Kursar, T. A. (2002). The effects of weather on fungal abundance and richness among 25 communities in the Intermountain West. *BMC Ecology*. https://doi.org/10.1186/1472-6785-2-7

Ter Halle, A., Drncova, D., & Richard, C. (2006). Phototransformation of the herbicide sulcotrione on maize cuticular wax. *Environmental Science and Technology, 40*(9), 2989–2995. https://doi.org/10.1021/ES052266H/SUPPL_FILE/ES052266HSI20060220_090243.PDF

Tesh, S. A., Tesh, J. M., Fegert, I., Buesen, R., Schneider, S., Mentzel, T., … Stinchcombe, S. (2019). Innovative selection approach for a new antifungal agent mefentrifluconazole (Revysol®) and the impact upon its toxicity profile. *Regulatory Toxicology and Pharmacology, 106*, 152–168. https://doi.org/10.1016/J.YRTPH.2019.04.009

Thacker, J. R. M., & Young, R. D. F. (1999). The effects of six adjuvants on the rainfastness of chlorpyrifos formulated as an emulsifiable concentrate. *Pesticide Science, 55*(2), 198-200. https://doi.org/10.1002/(sici)1096-9063(199902)55:2<198::aid-ps867>3.0.co;2-r

Théau, J., Gavelle, E., & Ménard, P. (2020). Crop scouting using UAV imagery: A case study for potatoes. *Journal of Unmanned Vehicle Systems, 8*(2). https://doi.org/10.1139/juvs-2019-0009

Tian, S., Yan, H., Meng, Z., Jia, M., Sun, W., Huang, S., … Zhu, W. (2022). Prothioconazole and prothioconazole-desthio induced different hepatotoxicities via interfering with glycolipid metabolism in mice. *Pesticide Biochemistry and Physiology, 180*, 104983. https://doi.org/10.1016/J.PESTBP.2021.104983

United States Department of Agriculture. (2021). *Blueberries Around the Globe – Past, Present, and Future*. Foreign Agricultural Service: International Agricultural Trade Report. https://www.fas.usda.gov/data/blueberries-around-globe-past-present-and-future

University of Hertfordshire. (2023). *Benzovindiflupyr*. Pesticide Properties DataBase. http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/2621.htm

Van Den Berg, F., Kubiak, R., Benjey, W. G., Majewski, M. S., Yates, S. R., Reeves, G. L., … Van Der Linden, A. M. A. (1999). Emission of pesticides into the air. *Water, Air, and Soil Pollution, 115*(1–4), 195–218. https://doi.org/10.1023/A:1005234329622/METRICS

Vander Kloet, S. (1988). *The genus Vaccinium in North America*. Agriculture Canada: Ottawa, ON.

Voegele, R. T., Wirsel, S., Möll, U., Lechner, M., & Mendgen, K. (2006). Cloning and characterization of a novel invertase from the obligate biotroph and analysis of expression patterns of host and pathogen invertases in the course of infection. *Molecular Plant-Microbe Interactions, 19*(6),625-634. https://doi.org/10.1094/MPMI-19-0625

White, S. N., Boyd, N. S., & van Acker, R. C. (2012). Growing degree-day models for predicting lowbush blueberry (*Vaccinium angustifolium* Ait.) ramet emergence, tip

dieback, and flowering in Nova Scotia, Canada. *HortScience, 47*(8), 1014-1021. https://doi.org/10.21273/hortsci.47.8.1014

Webb, K. T., Thompson, R. L., Beke, G. J., & Nowland, J. L. (1991). *Soils of Colchester County, Nova Scotia. Report No. 19*. Nova Scotia Soil Survey. Research Branch Agriculture Canada. https://sis.agr.gc.ca/cansis/publications/surveys/ns/ns19b/ns19b_report.pdf

Weete, J. D., Abril, M., & Blackwell, M. (2010). Phylogenetic distribution of fungal sterols. *PLoS ONE.* https://doi.org/10.1371/journal.pone.0010899

Wichura, A., Brand, T., & Böhm, J. (2020). Occurrence of *Thekopsora minima* on highbush blueberries in Lower Saxony 2015–2016: prevalence, susceptibility of varieties and some aspects of infection biology. *Journal of Plant Diseases and Protection, 127*, 359–366. https://doi.org/10.1007/s41348-020-00326-w

Wise, K. (2016). *Fungicide efficacy for control of wheat diseases*. Purdue University Extension. https://www.extension.purdue.edu/extmedia/BP/BP-162-W.pdf

Wood, G. (2004). The wild blueberry industry-past. *Small Fruits Review.* https://doi.org/10.1300/J301v03n01_03

Wu, C., Zhang, L., Mao, L., Zhu, L., Zhang, Y., Jiang, H., … Liu, X. (2022). Sorption and degradation of prothioconazole and its metabolites in soils and water sediments, and its combinative toxicity to *Gobiocypris rarus*. *Chemosphere, 303*, 135282. https://doi.org/10.1016/J.CHEMOSPHERE.2022.135282

Wu, X., Dong, F., Xu, J., Liu, X., Wu, X., & Zheng, Y. (2020). Enantioselective separation and dissipation of pydiflumetofen enantiomers in grape and soil by supercritical fluid chromatography–tandem mass spectrometry. *Journal of Separation Science, 43*(11), 2217–2227. https://doi.org/10.1002/JSSC.201901332

Xavier, S. A., Elis de Mello, F., Prudente da Silva, H., Canteri, M. G., Koga, L. J., de Oliveira Negrão Lopes, I., & Godoy, C. V. (2021). Microtiter method to monitor *Corynespora cassiicola* and sensitivity of the pathogen to carbendazim, prothioconazole and pyraclostrobin. *Crop Protection, 144*. https://doi.org/10.1016/j.cropro.2021.105554

Xi, N., Li, Y., & Xia, X. (2022). A review of pesticide phototransformation on the leaf surface: Models, mechanism, and influencing factors. *Chemosphere, 308*, 136260. https://doi.org/10.1016/J.CHEMOSPHERE.2022.136260

Xu, C., Li, M., Zhou, Z., Li, J., Chen, D., Duan, Y., & Zhou, M. (2019). Impact of five succinate dehydrogenase inhibitors on DON biosynthesis of *Fusarium asiaticum*, causing fusarium head blight in wheat. *Toxins, 11(5),* 272. https://doi.org/10.3390/toxins11050272

Zhang, B., Pan, X., Yang, Y., Dong, F., Xu, J., Wu, X., & Zheng, Y. (2023). Dissipation dynamics and comparative dietary exposure assessment of mefentrifluconazole in rice. *Ecotoxicology and Environmental Safety, 250*. https://doi.org/10.1016/J.ECOENV.2022.114482

APPENDICES

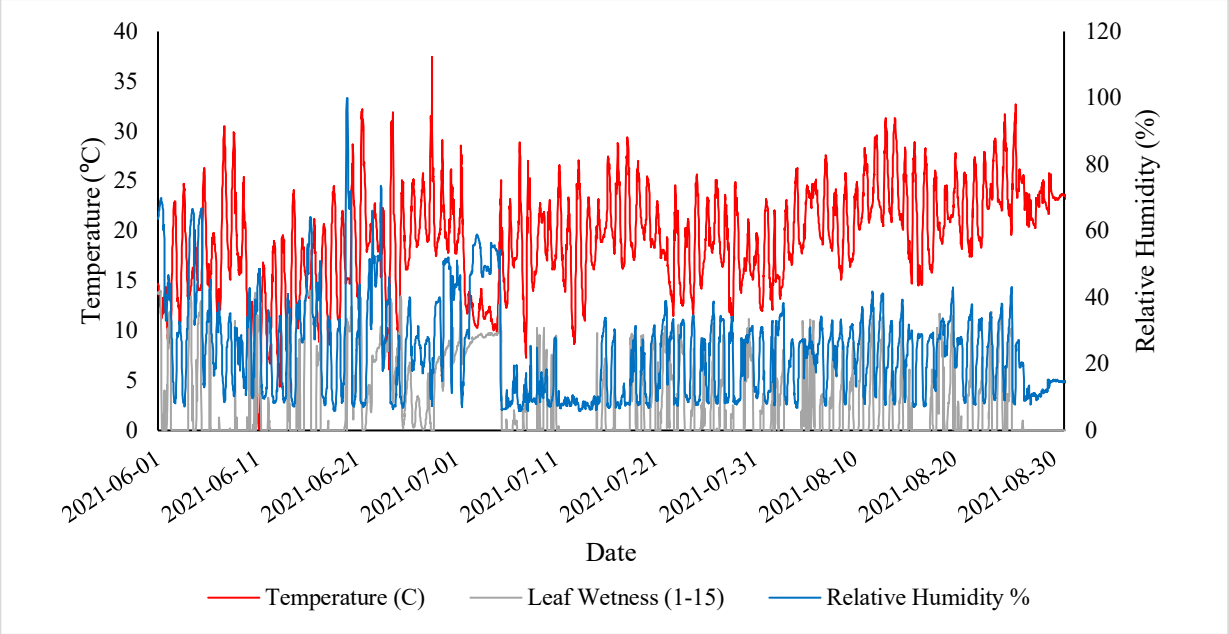

Figure A-1. Environmental conditions (temperature, relative humidity and leaf wetness) observed at a wild blueberry field near the Kemptown, NS field location (Mt. Thom; 10 km away from Kemptown). Data was collected on a Spectrum Technologies Watchdog 2700 weather station. Rainfall data was not collected on the weather station.

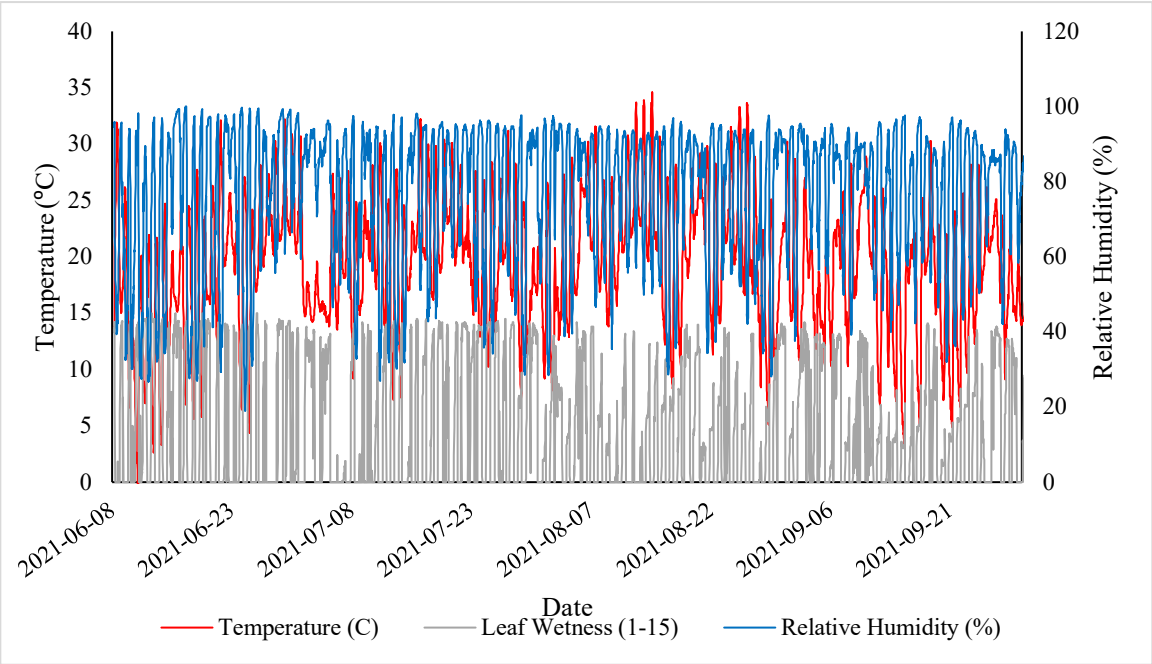

Figure A-2. Environmental conditions (temperature, relative humidity and leaf wetness) observed at the Wild Blueberry Research Center in Debert, NS. Data was collected on a Spectrum Technologies Watchdog 2700 weather station. Rainfall data were not collected on the weather station.

Table A-1. Kemptown environmental conditions corresponding with moderate and high risk potential infection periods for the months of June, July and August 2021 (Abbasi et al., 2022; Gupta et al., 2017; Pfister et al., 2004).

Start of Infection Period (Date, Time)	Leaf Wetness Duration (hrs)	Mean Relative Humidity (%)	Mean Temperature (°C)	Infection Period Rating
2021-06-20 0:00	35	35.8	18.4	high
2021-06-21 18:00	160	27.8	19.4	high
2021-06-28 17:00	162	43.1	16.2	high
2021-07-18 9:30	15	20.9	18.6	moderate
2021-07-20 10:30	13	27.3	18.8	moderate
2021-07-21 12:00	32	29.9	16.1	high
2021-07-23 2:00	19.5	23.8	15.5	moderate
2021-07-29 22:30	20	28.3	17.1	moderate
2021-08-01 23:30	21	32.2	15	moderate
2021-08-04 14:30	32.5	25.1	22.2	high
2021-08-18 5:00	44	30.7	21.3	high
2021-08-24 5:30	14.5	31.6	23.3	moderate

Table A-2. Debert environmental conditions corresponding with high risk potential infection periods for the months of June, July, August and September, 2021 (Abbasi et al., 2022; Gupta et al., 2017; Pfister et al., 2004).

Start of Infection Period (Date, Time)	Leaf Wetness Duration (hrs)	Mean Relative Humidity (%)	Mean Temperature (ºC)	Infection Period Risk Rating
2021-06-15 15:30	18	96.6	16.1	high
2021-06-21 23:30	16	94.2	20.8	high
2021-06-28 14:30	20.5	90.5	24.4	high
2021-07-02 3:30	30	90.5	15.5	high
2021-07-04 8:30	24	92.2	14.9	high
2021-07-18 19:30	15.5	92.4	20.0	high
2021-07-21 19:00	38.5	88.4	20.4	high
2021-07-29 20:30	36	88.6	16.3	high
2021-08-02 12:30	21.5	91.9	15.7	high
2021-08-04 21:00	20.5	89.7	18.9	high
2021-08-05 20:00	16.5	86.7	26.1	high
2021-08-18 17:30	18	89.5	19.9	high
2021-08-19 19:30	20	87.9	24.4	high
2021-08-30 10:00	24	91.2	20.5	high
2021-09-01 20:30	36.5	89.5	17.9	high
2021-09-05 22:00	34.5	88.6	19.1	high
2021-09-08 20:00	19.5	86.9	18.8	high
2021-09-09 17:30	40	85.1	24.2	high
2021-09-17 17:00	16.5	86.7	20.7	high
2021-09-26 4:30	28.5	85.9	21.9	high

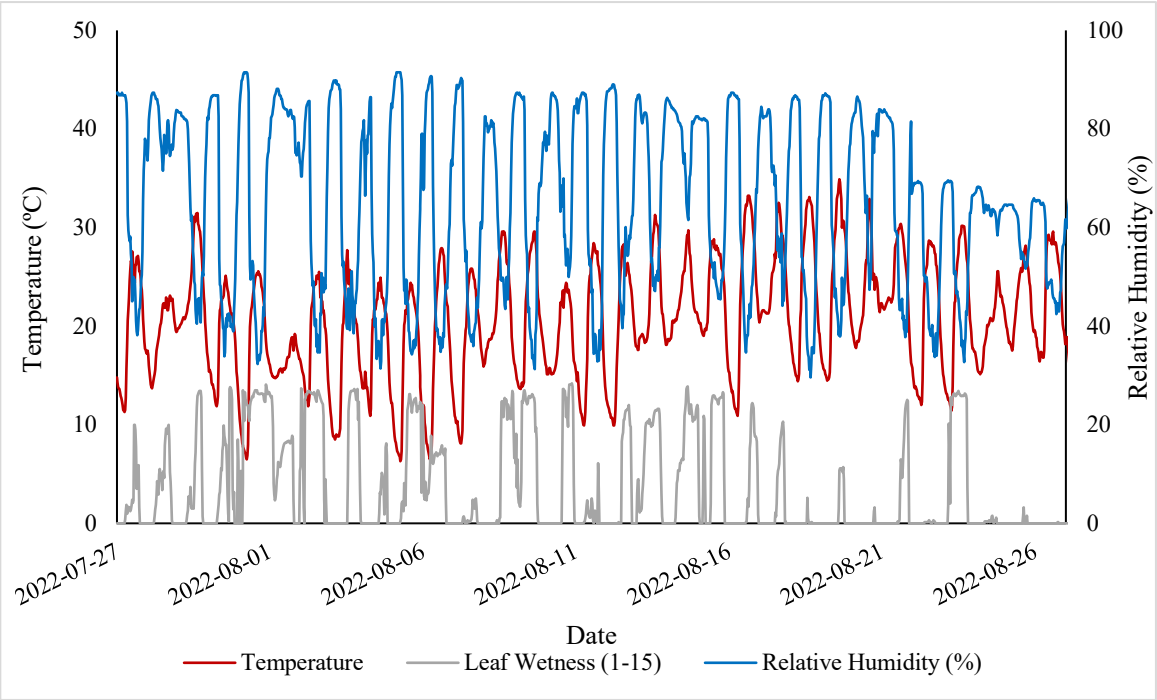

Figure A-3. Environmental conditions (temperature, relative humidity and leaf wetness) observed at the Wild Blueberry Research Center in Debert, NS. Data was collected on a Spectrum Technologies Watchdog 2700 weather station from July 27, 2022 to August 27, 2022 for the duration of the fungicide persistence study. Rainfall data were not collected on the weather station.