FUNGICIDAL SELECTION PRESSURE ON *MONILINIA VACCINII-CORYMBOSI* (READE) HONEY IN WILD BLUEBERRY (*VACCINIUM AUNGUSTIFOLIUM* AITON)

by

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ABSTRACT

Propiconazole has been extensively used throughout the wild blueberry industry for Monilinia blight control over the past 20 years. Given the apparent loss of Monilinia blight control observed in field research trials and also in commercial fields, concerns were raised that the natural population of *M. vaccinii-corymbosi* (causal agent of Monilinia blight) was becoming less sensitive to propiconazole. Combined with this was uncertainty of the sensitivity of *M. vaccinii-corymbosi* to other fungicides. Thus, laboratory sensitivity analysis of *M. vaccinii-corymbosi* isolates to propiconazole, difenoconazole, prothioconazole-desthio, boscalid, penthiopyrad, and cyprodinil was completed. Field trials were set up to investigate Monilinia blight control efficacy of different fungicides. A significant shift toward reduced sensitivity to propiconazole in the population of *M. vaccinii-corymbosi* was not detected. Prothioconazole-desthio was the most effective active ingredient to inhibit mycelial growth of *M. vaccinii-corymbosi*. Consistently, Tilt® (a.i. propiconazole) and Proline® (a.i. prothioconazole) showed significant Monilinia blight control *in vivo*.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ANOVA – Analysis of Variance
a.i. – Active ingredient
AP – Anilino-pyrimidine
APVMA – Australian Pesticide and Veterinary Medicines Authority
cm – Centimeter
DMI – Demethylation inhibitor
EC_{50} – Effective concentration that inhibits mycelial growth by 50%
g – Gram
h – Hour
ha – Hectare
JMPR – Joint FAO/WHO Meeting on Pesticide Residues
kg – Kilogram
kPa - Kilopascal
L – Liter
m – Meter
mg – Milligram
min – Minute
mL – Milliliter
mm – Millimeter
N – North
PDA – Potato dextrose Agar
pH – Decimal logarithm of the reciprocal of the hydrogen ion activity
PSI – Pound-force per square inch
QoI – Quinone outside inhibitor
r – Coefficient of correlation
RH – Relative Humidity
s – Second
SBI – Sterol biosynthesis inhibitor
SDHI – Succinate dehydrogenase inhibitor
spp. – Species
TM – Trade Mark
USEPA – United States Environmental Protection Agency
VF – Variation factor
W – West
µg – Microgram
µm – Micrometer
ºC – Degrees Celsius
® - Registered trademark
$ - Dollar
% - Percent
± - Margin of error of a quantity
< - Less than
> - More than

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Wild blueberry is lowbush blueberry, which has extremely long-lived clonal organism and is considered to be a primarily outcrossing woody perennial in the family Ericaceae (Vander Kloet, 1978). Wild blueberry fields are not sown, planted, or propagated from known genetic stock. They originate from wild seeds during reclamation of abandoned lands in North America. The individual plants of unknown genetic origins reproduce both sexually by bee-pollinated hermaphroditic flowers and clonally through an extensive underground rhizome system (Yarborough, 2015). These individual genotypes are referred to as "clones" because they are visually distinctive. The clones formed mosaic patches and can nearly completely cover the landscape due to the frequent burning and/or clearing of lands for several centuries. Two predominant species in northeastern North America are *Vaccinium. angustifolium* Aiton and *Vaccinium. myrtilloides* Michaux (Yarborough, 2015). Nowadays, most of the wild blueberry fields have been commercially managed on a biennial production cycle (commercially managed wild blueberry fields are referred to "commercial wild blueberry fields"). The plants are pruned every other year to maximize floral bud initiation, fruit set, yield and ease of mechanical harvest (Percival and Sanderson, 2004). The pruning is followed by vegetative growth of the plants for one year, which is the sprouting year. Swelling of buds, leaf expansion, bloom, pollination and berry harvest occurs in the following year, which is the crop year (Hall et al., 1979; Eaton et al., 2004b). Nova Scotia is a major

producer with commercial production exceeding 20 million kg in 2012, with a farm gate value in excess of $33 million.

Monilinia blight, caused by the pathogenic fungus *Monilinia vaccinii-corymbosi* (Reade) Honey, infects blueberry species, such as *V. angustifolium* (sweet lowbush blueberry), *V. myrtilloides* (sour top lowbush blueberry), and *V. corymbosum* L. (highbush blueberry). *M. vaccinii-corymbosi* belongs to the family of Sclerotiniaceae and the division of Ascomycota. The fungus overwinters in the form of pseudosclerotia (mummy berries) that fall from infected plants in the previous season. Pseudosclerotia start to germinate and form apothecia in the early spring. Mature apothecia are cup-like structures that produce sexual ascospores. Under favorable conditions, the ascospores cause primary infection on susceptible buds of wild blueberry (Batra, 1983; Munda, 2011). Infected leaves become water-soaked and infected blossom clusters become dark purple-brownish in colour and shrivel (Delbridge and Hildbrand, 1995). Asexual spores (conidia) are produced on infected tissues. Secondary infections occur when the conidia are dispersed onto the stigmas and infect ovaries (Batra, 1983). The infected berries become soft, light cream to salmon pink in colour and then turn light brown or greyish white and become wrinkled (Delbridge and Hildebrand, 1995).

M. vaccinii-corymbosi infection occurs when: fungal sporulation is taking place, during a 2- to 3- week period in early May; the buds are at susceptible stages of development and; there is an onset of precipitation and conducive temperatures. The disease can be destructive under prolonged wet conditions (i.e. several weeks of rain) (Delbridge and Hildebrand, 1995; Annis, 2009). The fungicide active ingredients triforine, propiconazole, metconazole and prothioconazole, demethylation inhibitors

(DMIs), are presently registered for Monilinia blight control in Canada. DMI fungicides have been extensively used for Monilinia blight disease suppression for approximately 30 years, since the first application of triforine in the 1980s (Lockhart et al., 1983). Because of development of fungicide resistance and residue issues within triforine in US and Europe, there is an export restriction on blueberries sprayed with triforine. For the past 20 years, wild blueberry growers in Nova Scotia have been reliant on propiconazole instead of triforine. Generally, application of propiconazole does not occur until after an infection period has occurred (D. Percival, personal communication, Dalhousie University, NS, CA). Reduced sensitivity of *Monilinia fructicola* (G. Winter) Honey to propiconazole has resulted from indiscriminate use of the fungicide (Zehr et al., 1999). Similarly, inconsistent and erratic control of Monlinia blight has been obtained in field trials conducted in 2007, 2008 and 2010 in Nova Scotia (Percival and Beaton, 2012). Recent fungicide efficacy and phytotoxicity trials carried out in Nova Scotia have indicated that prothioconazole can provide superior control of Monilinia blight, while causing no apparent visual phytotoxicity symptoms and no residue concerns in harvested berries. New Monilinia blight control products are in the process of being incorporated into wild blueberry management. They are fungicides that contain the active ingredient difenoconazole, which is also a demethylation inhibitor which has been shown to have relatively effective Monilinia blight control in field research trials (D. Percival, personal communication, Dalhousie University, NS, CA). It is essential to have alternatives available to replace fungicide products that are at risk of de-registration and/or resistance development and to rotate applications of fungicides with different modes of action to reduce the risk of insensitivity and resistance development.

Thompson and Annis (2014) reported reduced sensitivity of *M. vaccinii-corymbosi* to propiconazole in commercially managed wild blueberry fields in Maine, US. However, it was likely not widespread and no resistant isolates were detected. No previous study has shown a sensitivity shift of *M. vaccinii-corymbosi* in the commercially managed wild blueberry fields in Nova Scotia. Therefore, in order to utilize the fungicides to their fullest potential, it is necessary to examine both *in-vitro* sensitivity of the fungus to various active ingredients with different modes of action and *in-vivo* Monilinia blight control efficacy of diverse fungicide products with different modes of action. Combined with regulatory and market challenges (deregistration of existing fungicide products in export markets), further evaluation of alternate products is needed.

1.2 Objectives

The objectives of the study were to:

1) Examine whether the *M. vaccinii-corymbosi* population in commercially managed wild blueberry fields in Nova Scotia has developed reduced sensitivity or resistance to active ingredient propiconazole *in vitro*;
2) Investigate antifungal activity of new demethylation inhibitors prothioconazole and difenoconazole and other active ingredients with different modes of action against *M. vaccinii-corymbosi in vitro*; and
3) Investigate *Monilinia* blight suppression efficacy of demethylation inhibitors propiconazole, prothioconazole, and difenocoanzole and other active ingredients, with varying modes of action in wild blueberry fields.

CHAPTER 2

LITERATURE REVIEW

2.1 Physiology and management of wild blueberry

Wild blueberry plants are initially established from wild seeds. The plant that develops from a seed is the mother plant, which spreads vegetatively by an underground rhizome system. The rhizomes periodically send up stems above the soil surface, along with the development of roots on the rhizomes. All stems and rhizomes produced from the mother plants are referred as "clones". A wild blueberry field consists of many clones with one clone generally covering an area of 7 to 23 m^2 (Yarborough, 2015). Individual clones are genetically diverse, having various levels of genetic loads. The individual plants among clones can be easily distinguished from each other visually. Phenotypic variation among clones, including morphological, phonological, and yield differences, is dramatic (Vander Kloet, 1978). The high level of variability in an established wild blueberry field can be observed by differences in leaf color, berry color and size, and growth habit (Burger-MacLellan and MacKenzie, 2004). In northeastern North America, the predominant wild blueberry species are *Vaccinium angustifolium* Ait. and *Vaccinium myrtilloides* Michx., (Eaton et al., 2004a; Yarborough, 2015). *Vaccinium angustifolium* represents approximately 80% of the wild blueberry plants in the fields in Nova Scotia (D. Percival, personal communication, Dalhousie University, NS, CA).

Wild blueberry, also called lowbush blueberry, is a perennial winter-deciduous low shrub with woody stems (average height 20 cm) in the family of Ericaceae. Most wild blueberry fields are commercially managed on a biennial production cycle by pruning the

stems close to the ground in alternate years. After pruning, comes the first or sprouting year of the two-year production cycle, during which new shoots develop and two distinguishable types of buds are produced on the shoots tips (Eaton et al., 2004b; Jensen and Yarborough, 2004). Vegetative (leaf) buds are small and narrow, located on the lower part of the stem, while the floral buds located on the upper part of the stem are rounder and larger and develop into inflorescence in crop year (Hall et al., 1979; Mclsaac, 1997). Between the prune and crop year, the stems are leafless and the buds remain dormant over the winter. Bud swelling, leaf expansion, flowering and fruit production occur during the crop year (Hall et al., 1979; Eaton et al., 2004b).

The wild blueberry is indigenous to North America and has been commercially developed in Maine, the Atlantic Provinces (Nova Scotia (NS), New Brunswick (NB), Prince Edward Island (PE) and Newfoundland (NL)) and Eastern Quebec (QC) (Jensen and Yarborough, 2004). There are 24,200 hectares of wild blueberries in Maine (Yarborough, 2015), 29,100 hectares in QC, 16,200 hectares in NS, 12,949 hectares in NB, and 3,642 hectares in PE (D. Percival, personal communication, Dalhousie University, NS, CA). Wild blueberry plants typically grow in acidic soil with pH 4 to 5.5 (Hall et al., 1979). Wild blueberry fields originate from forested areas, scrub land or agricultural land that was abandoned and colonized (Hall et al., 1979; Mclsaac, 1997).

Commercial wild blueberry fields are usually pruned close to ground level by burning with oil, gas or straw or by mechanical mowing. Pruning in late autumn after harvest promotes the growth of new shoots, maximizes floral bud formation, and increases berry yield potential and harvestability (Mclsaac and Reid, 2000). Insect pollination is generally required for wild blueberry and is adequately attained by placing

honeybee, bumblebee or native bee hives in the fields during the bloom period (Mclsaac and Reid, 2000; Yarborough, 2015). The insects can also play a role as vectors, transmitting inoculum of fungal pathogen *Monilinia vaccinii-corymbosi* (Reade) Honey from infected leaves to flowers and from inoculated stigmas to new flowers, when the primary infection of *Monilinia* blight has occurred under favorable weather conditions (Batra, 1983).

2.2 The life cycle and morphology of *M. vaccinii-corymbosi* (Reade) Honey

Species of *Monilinia* Honey (discomycete fungi) attack plants of the ericaceous genera *Vaccinium*, *Gaylussacia*, *Ledum*, and *Rhododendron* (Batra, 1983). *Monilinia vaccinii-corymbsoi* (Reade) Honey has a wide host range of *Vaccinium* species, while the other *Monilinia* species on Ericaceae are host specific. This is one of the characteristics that distinguishes *M. vaccinii-corymbosi* from the other *Monilinia* species. *M. vaccinii-corymbosi* overwinters in the form of pseudosclerotia or mummy berries that drop to the ground before or at harvest (Honey, 1936; Batra, 1983). Pseudosclerotia or mummy berries, converted from infected fruits, are primarily composed of masses of fungal tissue. Pseudosclerotia are dark brown and hollow and have an outer and inner black rind in cross section (Batra, 1983; Munda, 2011). Carpogenic germination of pseudosclerotia produces apothecia, the only source of primary inoculum, during late winter/early spring (late April to early May) in the Maritime Provinces (Lockhart et al., 1983). Apothecia are reddish brown to amber. The fruiting cups are discoid in shape, with a slightly crenate margin (Batra, 1983; Munda, 2011). Cylindrical asci are formed in the apothecial cups. Eight sexual ascospores, the primary inoculum, are produced in each ascus. These

ascospores are nearly equal in size, hyaline, and ellipsoid (Batra, 1983; Munda, 2011). Ascospores measure 16 – 18 µm x 9 – 10 µm (Batra, 1991). Apothecia on lowbush mummy berries are smaller than those on highbush mummy berries (diameter × height: 3 – 7 mm × 6 – 22 mm versus 6 – 13 mm × 10 – 34 mm) whereas the size of the ascospores is approximately similar (Lockhart, 1961). In spring (May), ascospores ejected from mature apothecia are dispensed by wind to the surface of the young green tissues of the vegetative and floral buds (Lockhart et al., 1983; Ramsdell et al., 1974). Primary infection occurs when the ascospores germinate and penetrate the leaf tissue under favorable environmental conditions. Infected young leaves and flower clusters become blighted and wilt, but symptoms do not appear until 10 to 17 days after infection (Lockhart et al., 1983).

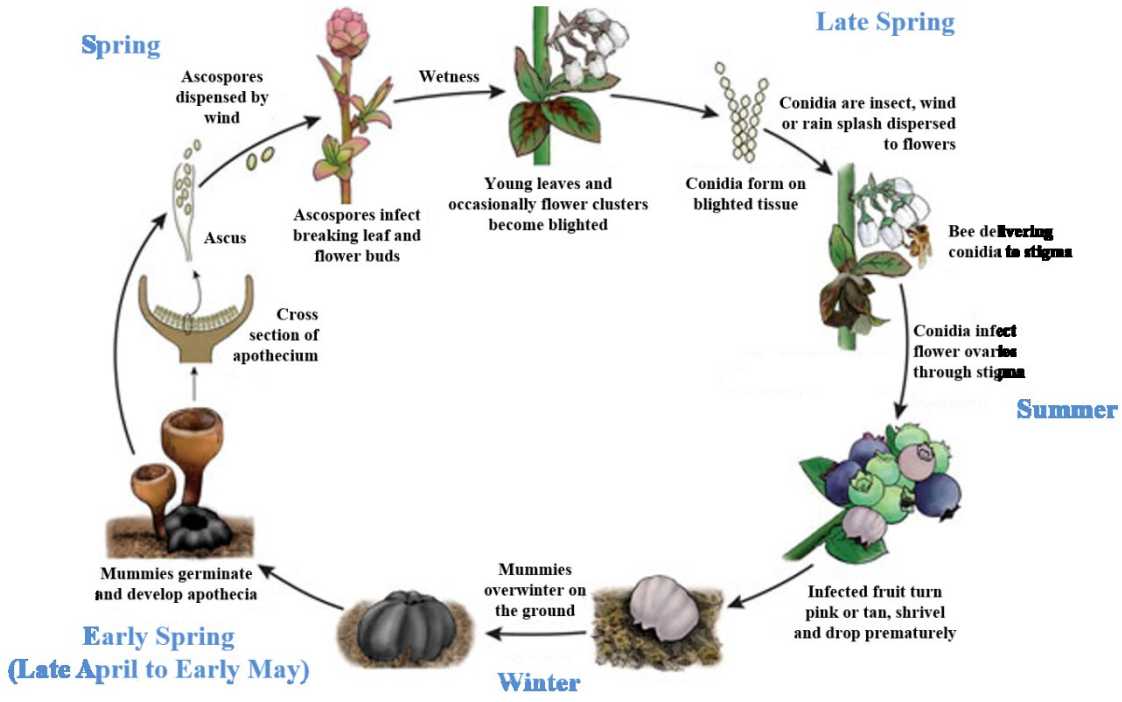


Figure 2-1 Disease cycle of mummy berry (Longstroth and Schilder, 2013)

In late spring (late May), grayish masses composed of conidiophores, conidia and occasional hyphae are produced on the upper surface of blighted shoots, peduncles, green bud scales, petioles and bases of the leaves, along the midrib (Batra, 1983). Conidia, the secondary inoculum, are asexual spores borne in long, branched or unbranched monilioid chains, within which individual conidia are separated by fusiform disjunctors (Batra, 1991; Munda, 2011; Honey, 1936). Individual conidia are limoniform (having a lemon shaped), hyaline and 26 – 28 μm × 19 - 21μm in the wild, 17 – 20 × 13 – 16 μm in culture (Batra, 1991). Conidia are disseminated by wind, rain splash and insects to the stigma of healthy flowers (Batra, 1983). Conidia germinate and grow through the stylar canal to the ovary in a manner similar to that of pollen tube growth, during which the growth of hyphae in the tissue of stigma, style and ovary causes the cells to collapse and die (Milholland, 1977; Shinners and Olson, 1996). In summer, the infected berries grow normally until they begin to ripen. As the infected berries are approaching maturity, the colour is initially light-cream and then turns to salmon pink (Batra, 1983). Ultimately, such berries turn light brown or grayish white and are converted into mummy berries or pseudosclerotia. The complete life cycle of *M. vaccinii-corymbosi* is shown in Figure 2-1.

2.3 Phenology of *M. vaccinii-corymbosi* and its infection on wild blueberry

The life cycle of *M. vaccinii-corymbosi* generally coincides with the growth development of its host plant (Figure 2-1). Lockhart et al. (1983) indicated that apothecial development of *M. vaccinii-corymbosi* occurred during the period of the initial growth of wild blueberry in Nova Scotia, and that ascospores were mature when the buds started to break. Batra (1983) reported that 60% of the lateral buds and 90% of the terminal foliar

buds had partly emerged from scales when the first mature apothecia were observed shooting ascospores. Bloom was 100% and blighted shoots were wilting when the last apothecia were ejecting ascospores and conidia were available for collection. Ascospore release lasts for approximately three weeks and ceases by the time floral buds bloom in the Maritime Provinces (Hildebrand and Braun, 1991). Lehman and Oudemans (1997) demonstrated that populations of *M. vaccinii-corymbosi* differ in timing of pseudosclerotium germination and apothecium development, which is proposed to be a component of fungal fitness to their original host cultivars and selected by host phenology. Lehman and Oudemans (2000) also concluded that the phenology of apothecium production is moderately to highly inheritable. Duration of conidium dispersal was reported to be 25 days in 1980 and 47 days in 1981 in a highbush blueberry field in Greenbelt, Maryland, during which healthy open flowers were ready for conidia infection (Batra, 1983).

Hildebrand and Braun (1991) described five stages of vegetative bud development and six stages of floral bud development of the blueberry host. The five stages of vegetative bud development were: V0 = dormant bud closed tightly; V1 = < 2 mm of green tissue emerged; V2 = 2 - 5 mm green tissue emerged; V3 = > 5 mm green tissue emerged and/or leaves beginning to separate; and V4 = leaves separating. The six stages of floral bud development were F0 = dormant bud closed tightly; F1 = buds swell, green tissue slightly visible; F2 = bud scales separating; F3 = sepal-covered flower buds visible; F4 = corolla visible in flower bud; F5 = corolla extending past calyx, pink or white bud pre-bloom. V2, V3, V4 and F2, F3, F4 are susceptible stages to *M. vaccinii-corymbosi* (Annis, 2009) (Figure A-1, A-2). Direct penetration into epidermal cells and indirect

penetration through stomates of highbush blueberry leaves were observed in the histopathology study of *M. vaccinii-corymbosi* conducted by Milholland (1977). Hildebrand and Braun (1991) also successfully observed germ tube penetration of *M. vaccinii-corymbosi* through stomata, without the formation of appressoria, on wild blueberry leaves. Greater numbers of stomata were observed at more advanced stages of bud development, whereas a few or no stomata were found on the exposed green tissues at the V1, V2, F1 and F2 stages of bud development. Higher disease incidence at more advanced stages of bud development was thought to be likely associated with increased stomatal density (Hildebrand and Braun, 1991).

2.4 Environmental conditions required for *M. vaccinii-corymbosi* infection to occur

As long as the apothecia, the only source of primary inoculum, successfully grow from the pseudosclerotia and discharge ascospores, there is a chance for ascosporic infection to occur. Factors affecting apothecial development include chilling hours, soil moisture, temperature, burial depth of pseudosclerotia and light (Milholland, 1974; 1977). A minimum of 900 to 1200 hours of exposure at 5 to 7 ºC is required to initiate germination and development of normal apothecia (Milholland, 1977). The sufficient soil moisture content for apothecial development ranges from 30 to 40% (Milholland, 1974). With adequate soil moisture, a temperature of 5 ºC is sufficient to stimulate germination of apothecia, while a temperature of 16 ºC is optimal for emergence and maturation (Milholland, 1974). Burying, or partial burying of pseudosclerotia can reduce emergence and apothecial development. A depth of 2.5 cm can inhibit apothecial development (Milholland, 1974). Light is required for the development of mature apothecia. Lockhart

et al. (1983) observed that mummy berries lying on fallen leaves or other debris did not develop apothecia. These mummy berries did not obtain sufficient soil moisture, which was thought to be the most important factor in germination and apothecial development (Milholland, 1974).

Relative humidity (RH) and temperature are considered to be the factors affecting ascospore release; rain and free moisture (leaf wetness) have no effect on the release of ascospores, and wind speed assists in dispersing the released ascospores, but has no effect on ascospore release (Ramsell et al., 1974). RH was found to have a significant inverse effect upon ascospore release. Mature apothecia eject ascospores primarily during periods of high humidity. Ascospore discharge is restricted in a saturated atmosphere (100% RH), but is greatly increased as RH is lowered. RH has been reported to be responsible for the circadian-like rhythm of ascospore discharge in the field. Alternate wetting and drying of the apothecium could rupture the inoperculate asci and thereby cause ascospore discharge to occur. Temperature has a highly significant direct effect on ascospore release. Wharton and Schilder (2005) illustrated that apothecia had greater initial ascospore discharge at higher temperatures (20 and 25 ºC), whereas total ascospore discharge was greater from apothecia at lower temperatures (10 and 15 ºC) where ascospore discharge persisted longer.

After successfully landing on the surface of the susceptible young green tissue of the blueberry buds, the ascospores germinate and penetrate to the plant tissue under favorable environmental conditions, causing primary infections to occur. The ascospore is thought to germinate over a wide range of temperatures. Ramsdell et al. (1974) reported that ascospores germinated within 6 hours in distilled water at 5 to 20 ºC and 15

ºC was the optimum temperature for ascospore germination and germ tube growth under laboratory conditions. Milholland (1977) observed numerous ascospore germ tubes on inoculated leaves at room temperature (20 to 25 ºC). Milholland (1977) also observed direct penetration into an epidermal cell and indirect penetration through stomata 48 hours after inoculating ascospores on highbush blueberry leaves at 20 to 25 ºC. Adequate moisture (prolonged leaf wetness) and conducive temperatures favors the ascospore germination, germ tube growth and invasion of the plant tissues, resulting in primary infection (Hidebrand and Braun, 1991). The risk of *M. vaccinii-corymbosi* infection is based on leaf wetness duration and temperature (Table 2-1). If the temperature is lower, a longer leaf wetness period is required for the infection to occur (Hildebrand and Braun, 1991).

Successful ascosporic (primary) infections lead to conidial (secondary) infections. Ramsdell et al. (1974) reported conidial dispersal was directly correlated with wind speed, whereas free moisture (dew) inhibited conidium release, and relative humidity and temperature had no effect on conidium release. The presence of disjunctors between individual conidia function as a separating mechanism, which is a special adaptation for wind dissemination of conidia (Honey, 1936). The greyish conidia masses on infected tissue produce free sugars and reflect UV light similar to blueberry floral calyces that attract insects and thus, the insects play a role as vectors, transmitting conidia from blighted tissues to flower stigmas and from inoculated stigmas to new flowers (Batra, 1983; Batra and Batra, 1985).

Table 2-1 Risk of infection according to environmental conditions for *M. vaccinii-corymbosi* infection periods (Delbridge and Hildbrand, 1995; Annis, 2009)

	Mean temperature (ºC) during infection periods				
Wetness duration hours	2	6	10	14	18
2	None	None	None	None	None
4	None	None	None	Low	Mod
6	None	Low	Low	High	High
8	None	Mod	High	High	High
10	Mod	High	High	High	High
15	Mod	High	High	High	High
24	High	High	High	High	High

2.5 Symptoms of Monilinia blight or mummy berry

The disease caused by *M. vaccinii-corymbosi* is called Monilinia blight or mummy berry. The symptoms do not show until 10 to 17 days after the susceptible buds were infected by the ascospores (Delbridge and Hildebrand, 1995). Discoloration initially appears at the upper side of bent shoots, distally spreading into the midribs and lateral veins of leaves (Hildebrand and Braun, 1991). Leaf discoloration travels from the base upward and outward until the entire leaf becomes dark brown (appearing as water-soaked) and wilt (Batra, 1983). Discoloration on floral buds initially appears on the sepals and spreads downward to the ovary and peduncle and distally to the corolla stem (Hildebrand and Braun, 1991). Ultimately, all the flowers within a cluster become infected and turn dark purple-brown in color (Delbridge and Hildebrand, 1995). A white-greyish conidia mass is produced on the surface of the infected tissues except the corolla,

usually appearing on the upper surface of infected leaves along the midribs and at the base of infected blossoms (Hildebrand and Braun, 1991). When the berries are approaching maturity, the infected berries start to show the symptoms, initially appearing soft, cream to salmon pink (Batra, 1983). As the fungus invades outward, the berries eventually turn light brown or greyish white and become irregularly wrinkled, hardened, furrow-ribbed and hollow inside, which is called mummification (Batra, 1983). The mummy berries fall off the plants and overwinter on the ground. The skin of the resulting mummy berries sloughs off.

2.6 Forecasting and monitoring of *M. vaccinii-corymbosi* infection

Three factors are required to be present at the same time to cause the primary infection of *M. vaccinii-corymbosi* to occur: I) blueberry buds develop to susceptible stages (vegetative buds reach stage V2 and floral buds reach stage F2); II) apothecia eject ascospores which are dispersed onto the surface of the susceptible bud and; III) there is a prolonged period of wetness with conducive temperature (Table 2-1) (Annis et al., 2009).

The blueberry blog (http://www.novascotiawildblueberryblog.com) and hot line (phone line) are used by growers to track blueberry bud development. When 40 to 50% of buds have reached stage V2 (2 - 5 mm green tissue emerged) and F2 (bud scales separating), the field is considered to be susceptible to *M. vaccinii-corymbosi* infection (Delbridge and Hildebrand, 1995). If a field has a past history of *M. vaccinii-corymbosi* infection, the mummy berries are present in the field and are available to germinate and develop apothecia. To predict when apothecial cups start to discharge ascospores, mummy berry patches are set up in the field to track the progress of germination and

development of apothecium cups (Annis et al., 2009). However, the ascospores produced in the adjacent sprout fields could also be the source for primary infection because they can be carried by wind for 30 m (Cox and Scherm, 2001). Rain and fog are considered to be responsible for causing prolonged leaf wetness to initiate infection, while dew is usually not enough to initiate infection (Delbridge and Hildebrand, 1995).

Annis et al. (2009) and Annis and Yarborough (2014) indicated that this mummy berry forecasting method typically requires fewer fungicide applications, compared to the calendar method. Using the calendar method, the first fungicide spray is applied when some buds are open and the infection periods have not occurred. The second fungicide application is 7 to 10 days later. Following the mummy berry forecasting method, the first fungicide spray is not applied until an infection period has occurred. The second fungicide application is based on the weather. If the weather is sunny and dry until the day that the apothecial cups are no longer producing spores, it is not necessary to make a second fungicide application.

2.7 Monilinia blight control

Intense and uniform burning, as a pruning technique, can be effective in destroying mummy berries, reducing Monilinia blight pressure (Delbridge and Hildebrand, 1995). By contrast, flail mowing does not destroy mummy berries, so the levels of the disease are generally increased (Delbridge and Hildebrand, 1995). Lambert (1990) reported that a 90-fold increase in mummy berry disease was obtained over six crop cycles (12 years), during which mowing was used as the pruning technique. Mummy berry disease was reduced 2- to 3-fold after light burning was resumed in a previously mowed field

(Lambert, 1990). Sequential burning has high cost and reduces the organic matter content in the soil. It is more beneficial to burn the fields every second or third crop cycle, rotating with mowing (Delbridge and Rogers, 2013). Besides, efficient harvest techniques can reduce the number of infected berries dropping to the ground (Annis and Yarborough, 2014).

In Canada, *Bacillus subtilis* QST 713 (products: Serenade® MAX and Serenade® OPTI) is the only biological agent registered for Monilinia blight control (Delbridge and Rogers, 2013). *B. subtilis* is a spore-forming, gram-positive bacterium that has antagonistic activity against pathogens, excreting antibiotics and possibly triggering a host's natural defence mechanisms (Wulff et al., 2002). *B. subtilis* produces antifungal polypeptides that decrease both spore germination and hyphal growth (McKeen et al., 1986). McGovern et al. (2012) only found one field attained effective Monilinia blight control by applying a high rate of Serenade® MAX whereas eight fields were tested. Serenade® MAX and Serenade® OPTI also have efficacy issues according to research trials conducted in commercial wild blueberry fields in Nova Scotia (Dr. David Percival, personal communication, Dalhousie University, NS). Schem et al. (2004) demonstrated that *B. subtilis* QRD 137 significantly reduced the growth rate of *M. vaccinii-corymbosi* in blueberry styles *in vitro* and that the formulated product also significantly inhibited fungal ingress into stigmas of detached flowers (control conidia infection). However, ascosporic infection of floral buds usually causes the greatest yield loss in wild blueberry because the fungus can directly destroy all the flowers within a cluster. Severe ascosporic infection of the leaves is also thought to affect berry size (Hildebrand and Braun, 1991). Disease incidence greater than 30% blighted leaves reduces berry size (Penman and

Annis, 2005). Conidial infection of lowbush blueberry has less impact on the yield, though it is still important for the mummification of the berries (Hildebrand and Braun, 1991).

In crop management, pesticides should be the last choice for controlling diseases. Reducing pesticide usage decreases environmental risk and lowers input costs. Fungicides are applied when and where needed to obtain desired level of control. Burning reduces the number of mummy berries, but may not give sufficient control of disease. Most fungicides are effective at protecting, but not curing diseased plants. Protective fungicide applications can lead to good control. The timing of the first fungicide application is crucial, since primary infection causes the most of the damage in wild blueberries, and conidia are produced on primary-infected plant tissues, leading to secondary infection. The first fungicide application should be made when 40 to 50% of the buds have developed to stage V2 and F2 (buds are susceptible to *M. vaccinii-corymbosi*) and the infection period has occurred (prolonged wetness period with conducive temperature) (Delbridge et al., 1998). According to Milholland (1977), the hypha produced from ascospores start to penetrate into leaf tissue 48 hours after inoculating ascospores on highbush blueberry leaves at 20 to 25 ºC. If a frost has occurred, the susceptibility of the buds will be greatly increased, and this condition will last for approximately four days (Delbridge and Hildebrand, 1995).

The active ingredients presently registered for Monilinia control in wild blueberry in Canada include: propiconazole (Tilt® 250E, Mission® 418EC, and Jade™), metconazole (Quash™), triforine (Funginex® 190EC), fluazinam (Allegro® 500F), penthiopyrad (Fontelis®), azoxystrobin (Quilt®) and prothioconazole (Proline® 480SC)

(Delbridge et al., 2015). Demethylation inhibitors (DMIs) are the main products used to control Monilinia blight in Nova Scotia. Propiconazole, metconazole, triforine, and prothioconazole are DMIs, where propiconazole is the most commonly used fungicide for Monlinia blight control in Nova Scotia. Wild blueberry growers have been reliant on propiconazole for over 18 years. Propiconazole is a mixture of cis-trans isomers, both exerting biological activity (European Food Safety Authority, 2010). Propiconazole can slow or stop the growth of the fungus, effectively preventing further infection and/or invasion of host tissues (European Food Safety Authority, 2010). Metconazole is also a mixture of cis-trans isomers, of which the cis-isomer is the main constituent in the technical active substance (European Food Safety Authority, 2011). Metconazole has no effect on fungal spore germination, but inhibits mycelial growth and prevents spore formation, showing both protectant and curative properties. Metconazole is also one of the most effective active ingredients for Monilinia blight control in highbush blueberries in the US (A. Schilder, personal communication, Michigan State University, Michigan, US). Prothioconazole is a systemic fungicide with protective, curative and eradicative activity (European Food Safety Authority, 2012). Prothioconazole has been proven to be an effective Monilinia blight control product (Percival and Beaton, 2012), with the additional benefit of no residues detected in harvested or processed berries.

DMIs are FRAC Group 3 fungicides (Fungicide Resistance Action Committee, 2015), belonging to the sterol biosynthesis inhibitors (SBIs). DMIs inhibit the enzyme cytochrome P450 sterol 14α-demethylase expressed by CYP*51* gene. DMIs were first introduced in mid 1970s and have been widely used for fungal disease control (Keith, 2012). These fungicides include chemical groups, such as piperazines (triforine), triazoles

(e.g., propiconazole, metconazole and difenoconazole) and triazolinthiones (prothioconazole) (Fungicide Resistance Action Committee, 2015). The first occurrence of fungicide resistance to DMI fungicides was observed in 1982 in *Sphaerotheca fuliginea* (Schlecht.) Pollacci and *Blumeria graminis* (DC.) Speer; they cause powdery mildew in cucurbit and barley, respectively. The resistance of pathogen to DMIs is described as "quantitative, multi-step or continuous", which means the pathogen populations manifest their resistance to DMI gradually, and are partial and variable in degree (Brent and Hollomon, 2007b). This is thought to be the result of polygenic resistance in the pathogen, which involves four major resistance mechanisms: 1) increased efflux of DMIs; 2) altered *Cyp51* gene, decreasing the binding of DMIs to the target site; 3) decreased demand for target-site product sterol 14α-demethylase and; 4) overexpressed *CYP51*, resulting in overproduction of sterol 14α-demethylase (Brent and Hollomon, 2007b; Leroux and Walker, 2011). Due to the polygenic resistance of the pathogen to DMI fungicides, if the DMI fungicide in question is much less used, its effectiveness will revert rapidly (Brent and Hollomon, 2007b).

Cross-resistance can occur between fungicides that belong to the same chemical group or have the same mode of action. If cross-resistance is present, the pathogen that develops resistance to one fungicide will automatically and simultaneously become resistant to the other fungicide (Brent and Hollomon, 2007b). Cross-resistance is not shown between the SBI groups, but is generally accepted to be present between DMI fungicides active against the same fungus (Fungicide Resistance Action Committee, 2015). A study by Holb and Schnabel (2007) observed cross-resistance among triazoles by noting their effects on *M. fructicola* on peach.

Monilinia species have a medium risk of developing resistance to fungicides (Fungicide Resistance Action Committee, 2013b). *M. fructigena* Honey*, M. fructicola* (G. Winter) Honey and *M. laxa* (Aderh, & Ruhland) Honey are listed as resistant pathogenic organisms to certain fungicides by FRAC (Fungicide Resistance Action Committee, 2013a). In a study by Luo and Schnabel (2008), an isolate of *M. fructicola* was considered DMI-resistant when the relative mycelial growth was equal or greater than 50% at the discriminatory dose of 0.1 $\mu g \cdot mL^{-1}$ propiconazole. Reduced sensitivity of *M. fructicola* isolates from commercial peach orchards in Georgia, US, with prolonged exposure to DMI fungicides, was reported by Schnabel et al. (2004) with the effective concentration of propiconazole (0.216 $\mu g \cdot mL^{-1}$) that reduced the fungal growth by 50% (EC_{50}), comparing to the baseline EC_{50} (0.02 $\mu g \cdot mL^{-1}$). In research performed by Thompson and Annis (2014), the propiconazole EC_{50} for conventionally managed fields (0.020 $\mu g \cdot mL^{-1}$) was significantly higher than the baseline EC_{50} (0.016 $\mu g \cdot mL^{-1}$) of isolates from from unmanaged areas. Resistance in *M. vaccinii-corymbosi* did not seem to be widespread in wild blueberry fields in Maine, US.

In the long term, it is necessary to implement resistance management strategies in order to conserve fungicide effectiveness. Extensive and consecutive use of a specific fungicide can lead to a sensitivity shift toward resistant populations of the pathogen to the fungicide (Brent and Hollomon, 2007b). Zehr et al. (1999) showed that reduced sensitivity of *M. fructicola* to propiconazole was attained because of prolonged exposure. Therefore, the use of best management practices to minimize the risk of fungicide resistance in blueberry fields is critical in minimizing losses associated with Monilinia blight. Best practices include: application of fungicides only when necessary; restricting

the number of applications per season; not applying fungicides to fields that have no previous history or symptoms of *Monilinia* and; not applying a fungicide when a frost has not occurred and the severity rating of *Monilinia* infection period is low (Delbridge and Hildebrand, 1995; Delbridge et al., 1998). In addition, it is important to use the recommended dose on the label as lowering the dose may enhance survival of less sensitive strains of the fungus (Brent and Hollomon, 2007b). Tank mixing, rotation or alternation of fungicides with different modes of action can also effectively reduce resistance development.

CHAPTER 3

SENSITIVITY OF *MONILINIA VACCINII-CORYMBOSI* TO PROPICONAZOLE

3.1 Introduction

Demethylation inhibitors (DMIs) are systemic fungicides that have the mode of action of inhibiting sterol biosynthesis in fungal membranes (Fungicide Resistance Action Committee, 2015). The target site of DMIs is the C14-demethylation of lanosterol, a biosynthetic step that occurs during the conversion of lanosterol to ergosterol, which is the final product of fungal sterol synthesis and a key component of cell membrane structure in most true fungi (kingdom Mycota) (Köller, 1992; Köller and Scheinplug, 1987). DMIs block C14-demethylation by preventing the cytochrome P450 monooxygenase enzyme 14α-demethylase from catalyzing the demethylation of lanosterol (Köller, 1988; Köller, 1992). The 14α-demethylase is encoded by the gene *cyp51*/*erg11* (Fungicide Resistance Action Committee, 2015). Membrane function of the fungus is disrupted by the steric effects of methylated ergosterol molecules within the cell membrane double-lipid layer (Köller, 1988; Köller, 1992). Thus, the growth of the fungus slows or stops, effectively preventing further infection and/or invasion of host tissues. As one group of the fungicides that have systemic activity and site-specific mode of action, DMI fungicides are subject to resistance problems and are considered to have medium risk of resistance development (Fungicide Resistance Action Committee, 2015). Their regular use may result in the development of reduced sensitivity or resistance in target fungus, leading to reduced effectiveness or loss of disease control when they are used. Resistance or reduced sensitivity to DMIs is known in various fungal species (Zehr et al.,

1999; Wong and Midland, 2007; Golembiewski et al, 1995; Köller et al., 1997). Reduced DMI sensitivity has been reported to develop gradually in fungal populations over time and exhibits a shift toward reduced DMI sensitivity *in vitro*, which is thought to account for the slow stepwise or quantitative resistance development of pathogen to DMIs (Brent and Hollomon, 2007b; De Waard et al., 1993; Georgopoulos, 1988). Accumulation of several mechanisms may be required to cause practical disease control difficulties (Brent and Hollomon, 2007a).

Monilinia blight is one of the most devastating fungal diseases in commercial wild blueberry production in the Atlantic Provinces of Canada and Maine, US, reducing the yield and post-harvest quality of berries (Hildebrand and Braun, 1991; Lockhart et al., 1983; Penman and Annis, 2005). It is believed that *Monilinia* spp. Have a medium risk of developing resistance to fungicides (Fungicide Resistance Action Committee, 2013b). *M. fructicola* has been reported to be resistant to DMI fungicides in Georgia and South Carolina (Schnabel et al., 2004), New York (Luo et al., 2008; Villani and Cox, 2011), and New Jersey (Burnett et al., 2010) due to extensive application of DMI fungicides. DMIs are the major fungicide products used for Monilinia blight control in Nova Scotia. *M. vaccinii-corymbosi* population in commercial wild blueberry fields in Nova Scotia has been exposed to DMI fungicides for approximately 30 years since the first usage of triforine in the 1980s (Lockhart et al., 1983). It was replaced by propiconazole in the 1990s.

As the most economically efficacious fungicide, the active ingredient propiconazole has been the key component against Monilinia blight in Nova Scotia for the last 20 years. Propiconazole is a racemic mixture of four separated cis- and trans-

diasteromers, all providing biological activity (European Food Safety Authority, 2010). The combination of all isomers exerts broad spectrum and high level of activity of propiconazole (Ambrus et al., 1987). Reduced sensitivity of *M. vaccinii-corymbosi* population to propiconazole in conventionally managed wild blueberry fields in Maine, US has been reported by Thompson and Annis (2014). Wild blueberry growers in Maine have been using approximately two applications of propiconazole-based fungicides to control Monilinia blight in each crop year since 1998. The use pattern of propiconazole in Nova Scotia is similar. As a consequence of exclusive and consecutive application of propiconazole, a study of potential development of resistance or reduced sensitivity of *M. vaccinii-corymbosi* population in commercial wild blueberry fields in Nova Scotia is required. Since unmanaged fields in Maine were wild areas of lowbush blueberries and not treated with any propiconazole containing fungicide, *M. vaccinii-corymbosi* populations in this type of fields were not exposed to propiconazole and thus, could be considered as a baseline of propiconazole sensitivity (Zehr et al., 1999; Russell, 2004).

3.2 Materials and methods

3.2.1 Isolation of *M. vacccinii-corymbosi*

Mummy berries and *Monilinia*-blighted shoots of blueberry plants from five commercial wild blueberry fields in Nova Scotia were used for isolation of *M. vaccinii-corymbosi*. Mummy berries were collected in 2011 and 2012 in Farmington, Parrsboro, and Mt. Thom. *Monilinia*-blighted shoots were cut off from the blueberry stems (one shoot per stem) in May in 2013 in Parrsboro, Mt. Thom, Noel, and Blue Mountain. After collection, mummy berries were stored in the refrigerator at approximately 4 °C and the

Monilinia-blighted shoots were stored individually in small plastic bags in the freezer at approximately -18 °C.

To isolate *M. vaccinii-corymbosi* from mummy berries, non-broken mummy berries were rehydrated by soaking them in sterilized distilled water. To sterilize the surface, mummy berries were completely immersed in the 20% ultra-bleach (Compliments, Mississauga, ON, CA) for 3 to 5 min. The surface-sterilized mummy berries were rinsed three times with sterilized distilled water and were then placed on sterilized paper towel to dry the surface. A flame-sterilized scalpel was used to cut open the mummy berries and cut out several tiny blocks of the white medulla in the center of the mummy berries, avoiding the black rind and the seeds. These tiny blocks of white medulla were placed on potato dextrose agar (PDA) plates for further mycelial growth and incubated at 22 ± 2 °C.

To isolate *M. vaccinii-corymbosi* from *Monilinia*-blighted shoots, the blighted leaf tissues were cut in pieces (roughly 25 mm^2) . These leaf pieces were soaked in 70% ethanol for 5 to 10 seconds, and were then immersed in 10% ultra-bleach for 30 to 40 s for surface sterilization. Surface-sterilized leaf pieces were rinsed with sterilized distilled water and were placed on sterilized paper towel to dry the surface. These surface sterilized blighted leaf pieces were placed on PDA plates for further mycelial growth.

PDA medium were amended with a mixture of 0.5 $mg \cdot mL^{-1}$ streptomycin sulfate and 0.5 $mg \cdot mL^{-1}$ penicillin to prevent bacterial contamination (Batra, 1983, Munda, 2011). All plates were placed in an incubator at 22 ± 2 °C in the dark (Batra, 1983, Thompson and Annis, 2014) until *M. vaccinii-corymbosi* colonies were observed on the medium. If there were other fungi growing on the medium, the colonies that were

confirmed to be *M. vaccinii-corymbosi* were sub-cultured on new PDA plates. Cultures obtained from one mummy berry or one shoot were considered to be one isolate

3.2.2 Single-spore isolation from spore suspensions

Mycelial plugs from the original culture of each isolate were transferred onto new PDA plates. Plugs were inverted so that the mycelium was touching the medium. The plates were incubated for approximately 7 days at 22 ± 2 °C to obtain fresh colonies. Healthy blueberry leaves from the shoots collected previously were cut in pieces. These leaf tissues were placed between two pieces of paper towel and were wrapped with aluminum foil for autoclaving. Sterilized leaf tissues were placed on the edge of the colonies to allow *M. vaccinii-corymbosi* to infect the leaf tissues. After one or two days of incubation, the infected leaf tissues were transferred and spread onto V8 juice (Cambell Soup Co., Camden, US) agar medium (200 mL V8 juice, 3 g calcium carbonate, 15 g agar, and 800 mL distilled water). These plates were incubated at 15 ± 2 °C with a 12-h dark/light cycle. After 2 to 7 days, *M. vaccinii-corymbosi* sporulated on the infected leaf tissues, producing asexual conidia. Alternatively, sterilized leaf tissues were spread on V8 juice agar. Inverted mycelial plugs cut from the edge of colonies of fresh cultures (approximately 4 to 8 days old) were transferred onto the surface of the leaf tissues. Conidia were produced either on mycelium plugs or on infected leaf tissues. If some isolates did not sporulate on the leaf tissues or mycelium plugs, the procedures above were repeated until successful sporulation was attained.

A dissecting microscope was used to help in locating the conidia. The conidia were scraped off the leaf tissues or mycelial plugs and transferred to a 2-mL sterilized

centrifuge tubes that contained sterilized distilled water to obtain a conidial suspension. The sterilized distilled water was previously amended with Tween 20 (Sigma-Aldrich Co., St. Louis, US), which helped in separating individual spores. Since the conidia are moniliform, each tube containing conidia was placed on a Vortex Genie (Scientific Industries, Inc., Bohemia, NY, US) to separate the conidia. The concentration of the spores in each conidial suspension was measured with a hemocytometer.

To obtain single-spore isolates, a 1 spore/15 µL conidial suspension was made by diluting the original conidial suspension. Petri dishes (90 × 15 mm) containing 10 mL PDA were used for spore germination. Sixteen squares were marked at the bottom of the Petri dishes. Ten microliters of a 1 spore/15 µL conidial suspension was transferred onto each square by using an Eppendorf pipette so that most of the squares had only one spore on the medium. These plates were sealed with parafilm and placed on the bench at room temperature for approximately 24 h. A compound microscope was then used to observe whether the spores were germinating. The plates were inverted and placed under the microscope. The germinated spores with ideal length of hypha were circled with a permanent marker at the bottom of the plate, making sure only one spore was within the circle. Blocks of agar with one geminated spore were cut out and were transferred to new PDA plates for further mycelial growth. These single-spore plates were incubated at 22 ± 2 °C in the dark. One single-spore culture with prolific mycelial growth was selected for each isolate. All single-spore isolates obtained were named accordingly and were stored in the refrigerator at 4 °C.

3.2.3 *In-vitro* sensitivity test of *M. vaccinii-corymbosi* to propiconazole

A mycelial growth assay was used to test the sensitivity of *M. vaccinii-corymbosi* to propiconazole (Luo and Schnabel, 2008, Zehr et al, 1999). PDA medium was amended with analytical-grade propiconazole (Sigma-Aldrich Co., St. Louis, US). Propiconaozle was dissolved in acetone to make stock solutions, which were prepared on the day of testing. The stock solutions were diluted to different concentrations in order to make the final concentrations of propiconazole, which were 0, 0.003, 0.007, 0.01, 0.03, 0.07, 0.1 and 0.3 $\mu g \cdot mL^{-1}$. Unamended PDA medium was used as the control (Figure 3-1). Autoclaved PDA was cooled down to 50 °C before mixing propiconazole stock solutions with the medium. The final concentration of acetone was 0.1% (v/v). Each treated medium was poured in Petri dishes (90 × 15 mm). Two perpendicular lines were marked on the bottom of the Petri dishes (Figure 3-1). Mycelial plugs from the edge of the colonies were transferred and inverted on the intersection point of the two lines (Figure 3-1). All the plates were sealed with parafilm and were incubated at 22 ± 2 °C in the dark for approximately 7 days. Batra (1991) indicated that *M. vaccinii-corymbosi* had better mycelial growth on PDA in dark than in light. After a 7-day incubation, both horizontal and vertical diameters of the colony of each isolate were recorded. Each isolate was tested three times.

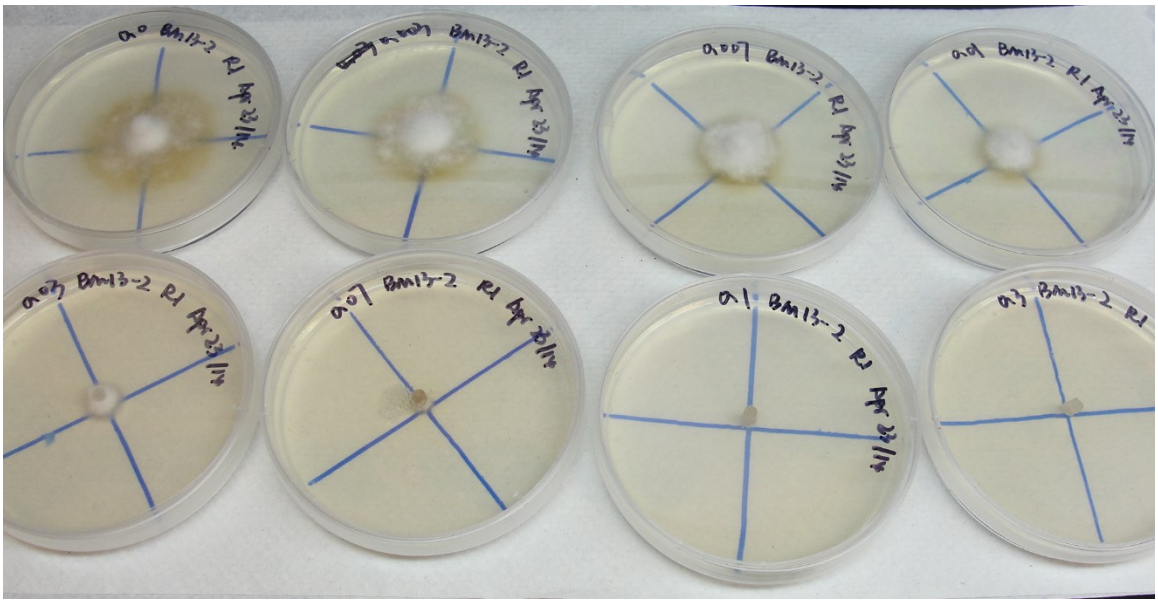


Figure 3-1 Mycelial growth of *M. vaccinii-corymbosi* on PDA amended with different concentrations of propiconazole after 7-day incubation at 22 ± 2 °C in the dark

3.2.4 Statistical analysis

The percent relative mycelial growth of each *M. vaccinii-corymbosi* isolate of each concentration in each replication was calculated by dividing the colony diameter on amended medium by the colony diameter on unamended medium and multiplying by 100. The colony diameter was calculated by subtracting the diameter of the plug from the average of the diameters recorded in two perpendicular directions (Figure 3-1). The effective concentration that inhibited the mycelial growth of each *M. vaccinii-corymbosi* isolate by 50% (EC_{50}) in each replication was calculated from the ensuing equation that was obtained by regression analysis of percent relative growth versus the propiconazole concentration using Sigmaplot (version 12.0, Systat Software Inc., San Jose, California, US) (Vinggaard, et al., 2002; Markey et al., 2007; Gachango et al., 2012). The average EC_{50} value determined from three replicates was used to express the propiconazole sensitivity level of each isolate. Propiconazole sensitivity of *M. vaccinii-corymbosi*

isolates collected from five different commercial wild blueberry fields in NS, CA was compared using one-way ANOVA by PROC ANOVA in SAS (version 9.4, SAS institute, Inc., Cary, NC). A two-sample t test by PROC TTEST in SAS (version 9.4, SAS institute, Inc., Cary, NC) was used to compare average propiconazole sensitivity between isolates from NS, CA and isolates from Maine, US.

3.3 Results and discussion

3.3.1 Morphological characteristics of *M. vaccinii-corymbosi*

Single-spore isolates varied in colony growth rate, compactness, pigmentation and sporulation of conidia on agar media. The mycelium was initially white and became beige or tan in older cultures (Figure 3-3-B). With further growth, the mycelium became more compact, with a cottony appearance (Figure 3-3-B, 3-3-C). The colonies were usually raised in the center and dense. The reverse sides of the plates were brown, with yellow or honey-coloured pigmentation (Figure 3-3-B). The colonies grew slowly on PDA or V8 juice medium. The average colony size on PDA was 3.6 cm after 7 days of incubation in the dark at 20 ± 2 °C. The maximum colony size was up to 5.5 cm after 7 days of growth. Brown stromata and thick, black hyphal fascicles developed in older cultures. Microconidia were abundant on agar media, and were apparent as small, hyaline, globose, and unicellular cells. Microconidia are usually borne directly on ascospores, conidia or hyphae (Batra, 1991, Munda, 2011). The asexual *M. vaccinia-corymbosi* microconidia initially observed on the agar media were nonviable, and these results are consistent those of Batra with macroconidia being approximately five times larger than microconidia, and having a far greater germination rate (Batra, 1991; Munda,

2011). Macroconidia production however, was initially scarce on agar media with no cells depicting the hyaline and lemon-shaped features. Macroconidia are usually formed by the budding of the terminal part of the conidiophores (Batra, 1991). Autoclaved blueberry leaf tissues placed on the medium facilitated sporulation of macroconidia. Macroconidia appeared as dense grayish mantles of conidiophores covering the leaf tissues (Figure 3-3-A). Macroconidia were borne in long, branched monilioid chains (Figure 3-3-D). Individual mature macroconidia were limoniform and hyaline and separated by fusiform disjunctors (Figure 3-3-F). Hyphae germinated from macroconidia were dichotomous, gnarled, broad and often assembled in fascicles (Figure 3-3-E, 3-3-G).

According to the previous morphological descriptions of *M. vaccinii-corymbosi* by Honey (1936), Batra (1983, 1988a, 1988b), and Munda (2011), the morphology of the fungus isolated from mummy berries and infected leaf tissues of wild blueberry in this study appeared to be similar to their observations, such as the appearance of colony on agar medium (Figure 3-2-A), shape of the conidia (Figure 3-2-D, 3-2-E) and the appearance of macroconidial chains (Figure 3-2-B). *M. vaccinii-corymbosi* is one of the *Monilinia* species that has disjunctors present (Figure 3-2-D, 3-2-E) (Honey, 1936; Batra 1983; Batra 1988b) and Batra (1988b) described the disjunctors of *M. vaccinii-corymbosi* in detail. The fungus isolated in this study had disjunctors present between individual mature macroconidia *in vitro* (Figure 3-3-F). Moreover, isolates sent to the National Fungal Identification Service Gestionnaire (Agriculture and Agri-Food Canada, Ottawa, ON, CA) for genomic analysis were detected to have similar sequencing to the isolate that has been proved to be *M. vaccinii-corymbosi* (unpublished data), but to be different

from the ITS sequencing results of *M. vaccinii-coyrmbosi* specimens from the study conducted by Holst-Jensen et al. (1997).

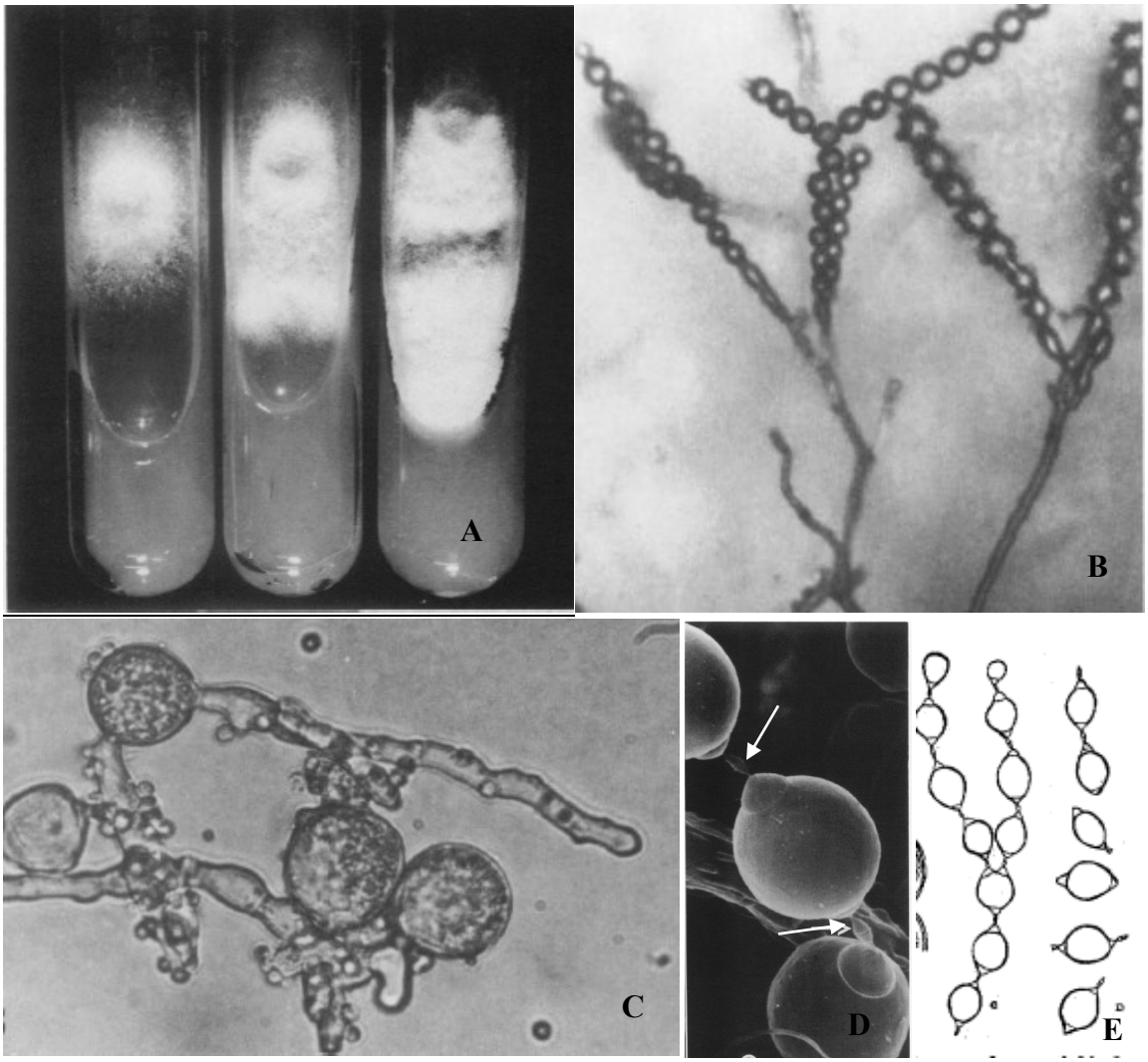


Figure 3-2 Literature descriptions of *Monilinia vaccinii-corymbosi*. A. 4-week old cultures of *M. vaccinii-corymbosi* on yeast malt agar (YMA), 12-h light/dark cycle, at 5, 12, and 20 C, respectively (Batra, 1988b); B. Conidial chains from *in-vitro* cultures (Batra, 1983); C. Germinating conidia (Batra, 1983); D. Portion of a conidial chain with two disjunctors (arrow) (Batra, 1988a); E. Diagrammatic representation of conidial chains found in the members of the section Disjunctoriae (Honey, 1963).

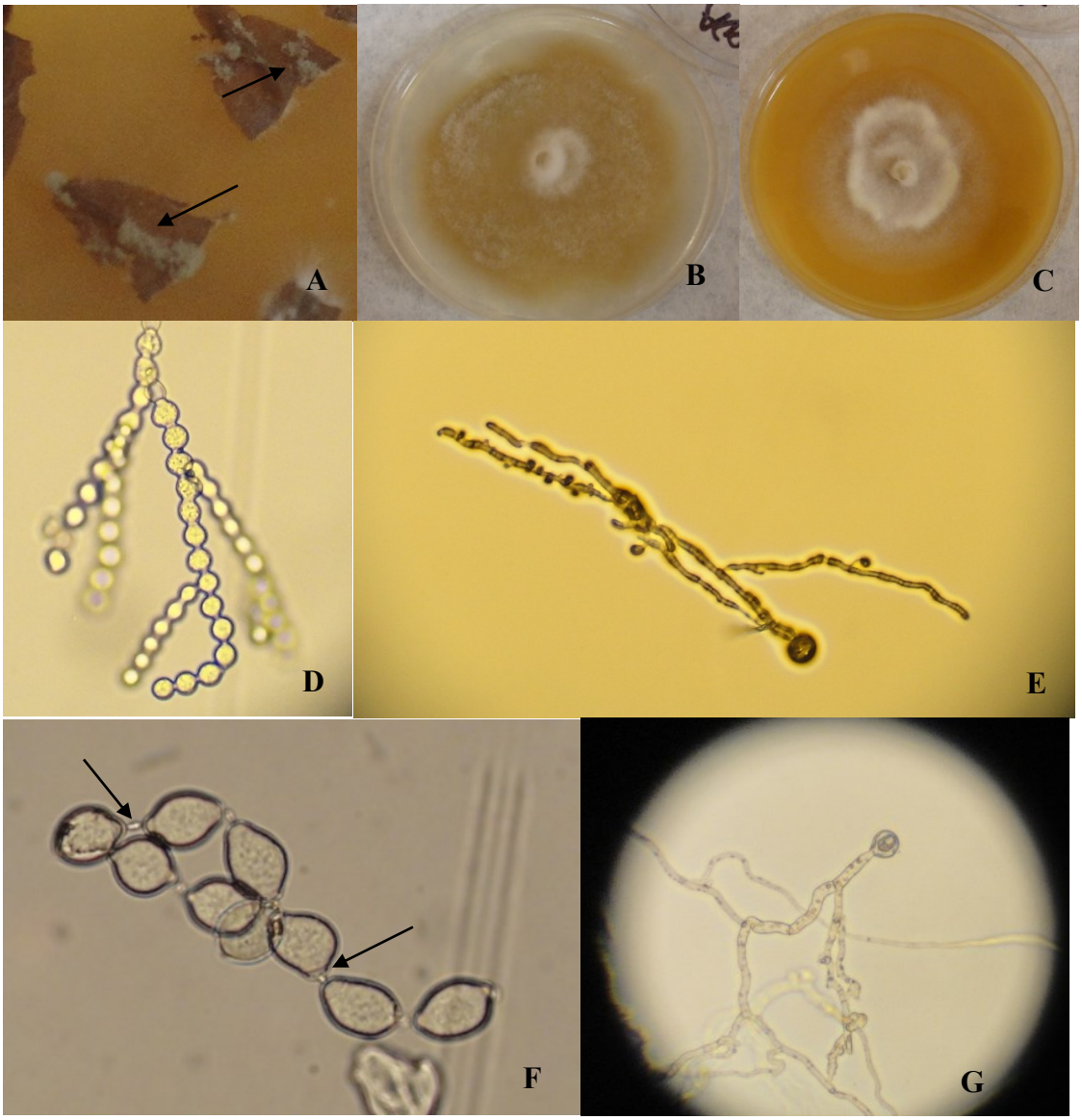


Figure 3-3. Morphological characteristics of *Monilinia vaccinii-corymbosi*. A. Conidial masses on blueberry leaf tissues (arrows); B. Colony of *M. vaccinii-corymbosi* on PDA; C. Colony of *M. vaccinii-corymbosi* on V8-juice agar; D. Monilioid macroconidial chains; E. Germinating macroconidia; F. Mature macroconidia connected with disjunctors (arrows); G. Hyphae of *M. vaccinii-corymbosi*.

3.3.2 *In-vitro* sensitivity of *M. vaccinii-corymbosi* to propiconazole

The total number of single-spore isolates tested was 102, of which 21 isolates were from Blue Mountain, 19 isolates were from Noel, 20 isolates were from Parrsboro, 20 isolates were from Mt. Thom, and 22 isolates were from Farmington. Propiconazole EC_{50} values of 102 isolates ranged from 0.007 to 0.036 $\mu g \cdot mL^{-1}$ with an average value of 0.015 $\mu g \cdot mL^{-1}$ (Table 3-1), which was not significantly different from the mean propiconazole EC_{50} value of 0.016 $\mu g \cdot mL^{-1}$ of baseline isolates and was significantly lower than the mean propiconazole EC_{50} value of 0.021 $\mu g \cdot mL^{-1}$ of isolates collected from conventionally managed fields in Maine (Table 3-2, 3-3). The frequency distribution of propiconazole EC_{50} values of 102 *M. vaccinii-corymbosi* isolates is shown in Figure 3-13F, appearing as a unimodal bell-shape, where 74.5% of the isolates had EC_{50} values less than 0.018 $\mu g \cdot mL^{-1}$, 15.7% of the isolates had EC_{50} values higher than 0.021 $\mu g \cdot mL^{-1}$, and 2.9% of the isolates had EC_{50} values higher than 0.030 $\mu g \cdot mL^{-1}$.

M. vaccinii-corymbosi isolates collected from five commercial fields exhibited different propiconazole EC_{50} values and frequency distributions. Isolates from Blue Mountain, Noel and Parrsboro, having mean EC_{50} values of 0.019, 0.016, and 0.016 $\mu g \cdot mL^{-1}$ respectively, were not significantly different from each other (Table 3-1). Both Mt. Thom and Farmington isolates had mean EC_{50} values of 0.013 $\mu g \cdot mL^{-1}$ that were significantly lower than the EC_{50} value of the Blue Mountain isolates (Table 3-1). Besides, Mt. Thom isolates and Farmington isolates had EC_{50} values that were significantly lower than the EC_{50} value of baseline isolates from Maine, US, while Blue Mountain, Noel, Parrsboro isolates had EC_{50} values that were not significantly different from baseline isolates (Table 3-3). According to the frequency distributions of

propiconazole EC_{50} values of Blue Mountain, Noel, Parrsboro, Mt. Thom and Farmington isolates, 52.4%, 73.7%, 70%, 85%, and 90.9%, respectively, of the isolates had EC_{50} values lower than 0.018 $\mu g \cdot mL^{-1}$; 33.3%, 26.3%, 20%, 0%, and 0%, respectively, of the isolates had EC_{50} values higher than 0.021 $\mu g \cdot mL^{-1}$; and 4.8%, 5.3%, 5.0%, 0%, and 0%, respectively, of the isolates had EC_{50} values higher than 0.030 $\mu g \cdot mL^{-1}$ (Figure 3-14 A, B, C, D, E).

On average, Nova Scotia *M. vaccinii-corymbosi* isolates had similar propiconazole EC_{50} values to baseline isolates from Maine. A significant shift toward reduced sensitivity to propiconazole in natural populations of *M. vaccinii-corymbosi* from commercial wild blueberry fields in Nova Scotia was not detected after regular exposure over the last 20 years. Nevertheless, 15.7% of Nova Scotia *M. vaccinii-corymbosi* isolates had propiconazole EC_{50} values higher than 0.021 $\mu g \cdot mL^{-1}$, where the isolates had higher risk of developing reduced sensitivity or even resistance to propiconazole under the selection pressure of propiconazole containing fungicides. Moreover, the highest EC_{50} value of 0.036 $\mu g \cdot mL^{-1}$ was close to the highest EC_{50} value (0.037 $\mu g \cdot mL^{-1}$) from conventionally managed fields in Maine, and was higher than the highest EC_{50} value (0.031 $\mu g \cdot mL^{-1}$) for baseline isolates (Table 3-2). The Blue Mountain field may have a higher risk of developing reduced sensitivity to propiconazole than the other fields, since 33.3% of the isolates had EC_{50} values higher than 0.021 $\mu g \cdot mL^{-1}$.

In-vitro sensitivity tests indicated that effectiveness of propiconazole was still maintained for Monilinia blight control. The life cycle of *M. vaccinii-corymbosi* may have contributed to the minor response under the propiconazole selection pressure. The complete life cycle of *M. vaccinii-corymbosi* requires sexual and asexual infection on

different plant tissues and, the overwintering in mummified berries. By contrast *M. fructicola* infects blossoms and twigs sexually and asexually at the same time and both mummified fruits and blighted tissues can produce conidia, which increases the number of generations and total number of spores per season. Therefore more extensive usage of fungicides is required to control the disease. *M. fructicola* has been reported to be resistant to DMI fungicides in Georgia and South Carolina (Schnabel et al., 2004), New York (Luo et al., 2008; Villani and Cox, 2011), and New Jersey (Burnett et al., 2010) due to extensive application of DMI fungicides. Additionally, prolonged wetness and conducive temperatures are also required for the infection to occur. Relatively dry weather can reduce the infection risk of *M. vaccinii-corymbosi* as well.

There is concern that the *M. vaccinii-coyrmbosi* population in commercial wild blueberry fields in Nova Scotia may have developed reduced sensitivity to propiconazole because of extensive and consecutive usage of propiconazole for approximately 20 years. Nevertheless, the *in-vitro* sensitivity test illustrated that propiconazole was effective in inhibiting mycelial growth of *M. vaccinii-corymbosi* isolates on amended PDA medium. The *M. vaccinii-corymbosi* population was still sensitive to propiconazole, and no resistant isolates were detected. It is a great advantage for wild blueberry growers in Nova Scotia that propiconazole can be continuously used as an effective and economical product for Monilinia blight control. To be vigilant, it is necessary to keep monitoring the sensitivity of *M. vaccinii-corymbosi* population to propiconazole. Moreover, it is also important to have other active ingredients with different modes of action available to rotate the applications, in order to avoid resistance development of propiconazole.

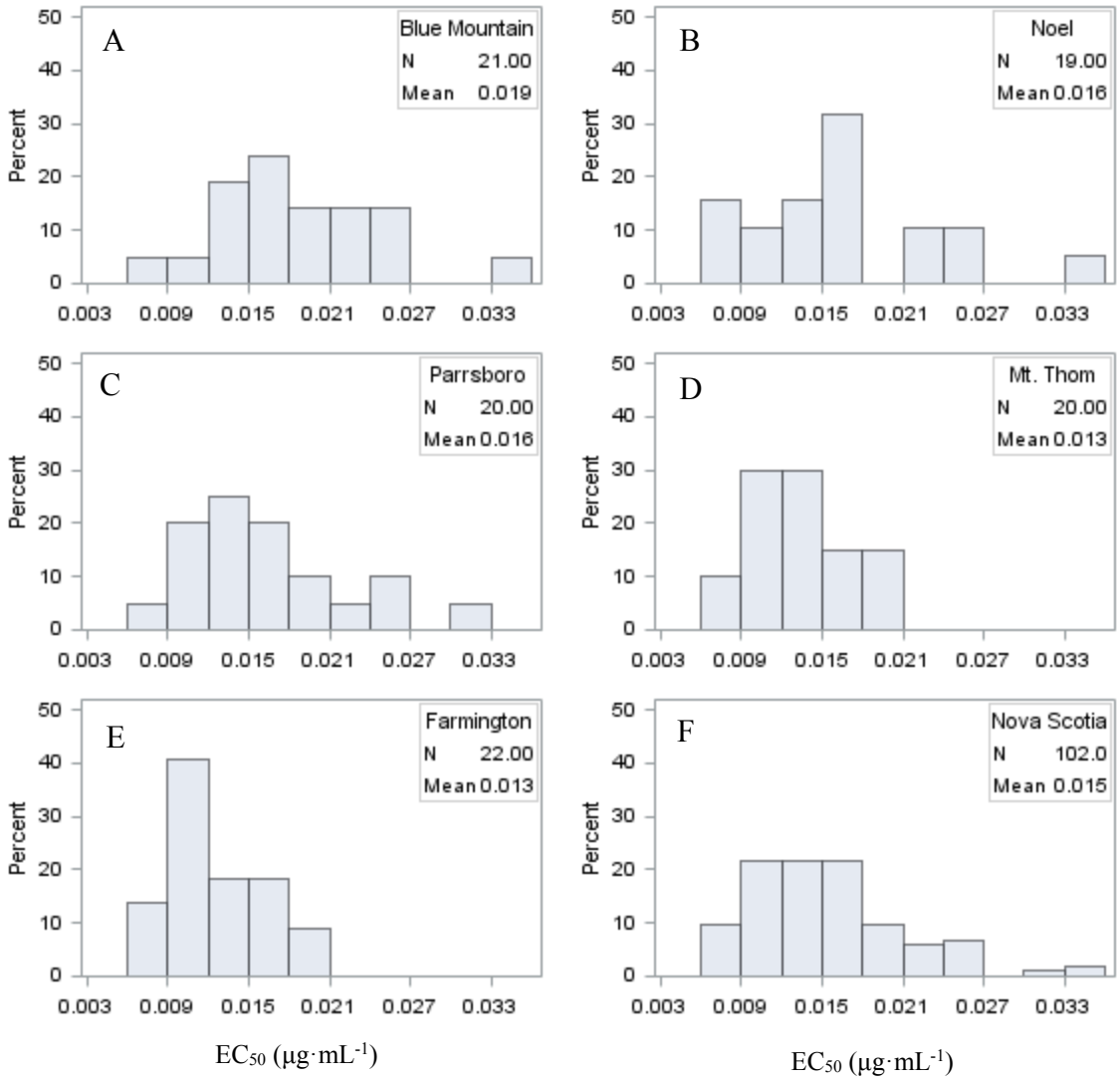


Figure 3-4 Frequency distribution of propiconazole EC_{50} values ($\mu g \cdot mL^{-1}$) for *M. vaccinii-corymbosi* isolates collected from 2011 to 2013 in commercial wild blueberry fields in Nova Scotia (A. Blue Mountain, B. Noel, C. Parrsboro, D. Mt. Thom, E. Farmington, F. All isolates collected in Nova Scotia). Individual isolates were grouped in class intervals of 0.003 $\mu g \cdot mL^{-1}$. The Figures were created by Proc UNIVARIATE procedure of SAS

Table 3-1 Characteristics of *M. vaccinii-corymbosi* isolates from commercial wild blueberry fields in NS, CA and their sensitivity to propiconazole

Origin of isolates				EC_{50} value ($\mu g \cdot mL^{-1}$)[a]		
Field	Year of collection	Source of isolation	No. of isolates	Range	Mean (± SEM)[b]	VF[c]
Blue Mountain	2013	Leaf tissue	21	0.008-0.036	0.019 (± 0.0013) *a*	4.5
Farmington	2011	Mummy berry	22	0.008-0.021	0.013 (± 0.0008) *b*	2.6
Mt. Thom	2012&2013	Mummy berry and leaf tissue	20	0.007-0.018	0.013 (± 0.0008) *b*	2.6
Noel	2013	Leaf tissue	19	0.007-0.034	0.016 (± 0.0015) *ab*	4.9
Parrsboro	2011&2013	Mummy berry and leaf tissue	20	0.008-0.031	0.016 (± 0.0014) *ab*	3.9
Nova Scotia (Total)	2011-2013	Mummy berry and leaf tissue	102	0.007-0.036	0.015 (± 0.00057)	5.1

[a] EC_{50} value is the effective concentration that reduces mycelial growth by 50%.

[b] Mean separation was completed using Tukey's mutiple means comparison test procedure ($\alpha<0.05$). Means sharing the same letter are not significantly different. SEM is the standard error of the mean.

[c] Variation factor (VF) = Highest EC_{50} value divided by lowest EC_{50} value.

Table 3-2 Propiconazole sensitivity of *M. vaccinii-corymbosi* isolates from wild blueberry fields in Maine, US (Thompson and Annis, 2014)

Field	No. of isolates	EC_{50} value ($\mu g \cdot mL^{-1}$)[a]		
		Range	Mean (± SEM)[b]	VF[c]
Conventional	39	0.012-0.037	0.021 (± 0.00098)	3.1
Unmanaged	16	0.011-0.031	0.016 (± 0.0012)	2.8

[a] EC_{50} value is the effective concentration that reduces mycelial growth by 50%.
[b] SEM is the standard error of the mean.
[c] Variation factor (VF) = Highest EC_{50} value divided by lowest EC_{50} value.

Table 3-3 Propiconazole sensitivity comparison between *M. vaccinii-corymbosi* isolates from commercial wild blueberry fields in NS, CA and isolates from wild blueberry fields in Maine, US

Fields		Two-sample *t* test *P*-values[a]					
		Isolates from NS (commercially managed)[b]					
		Blue Mountain	Noel	Parrsboro	Mt. Thom	Farmington	Nova Scotia (Total)
Isolates from Maine (Thompson and Annis, 2014)	Conventional[c]	0.18	0.013	0.008	<0.0001	<0.0001	<0.0001
	Unmanaged[d]	0.15	0.82	0.85	0.047	0.024	0.73

[a] Two means are significantly different from each other when *P*-value is less than 0.05 (α)
[b] Commercially managed: propiconazole was sprayed approximately twice for Monilinia blight control every crop year in Nova Scotia, CA
[c] Conventional: propiconazole was sprayed approximately twice for Monilinia blight control every crop year in Maine, US
[d] Unmanaged: propiconazole was never sprayed

CHAPTER 4

SENSITIVITY OF *M. VACCINII-CORYMBOSI* TO SIX ACTIVE INGREDIENTS WITH DIFFERENT MODES OF ACTION

4.1 Introduction

It is assumed that fungicide-resistant fungal mutants exist as subpopulations at a low, but finite, frequency before the exposure of the total population to a new fungicide (Skylakalis, 1987; Hollomon, 2015). Resistant mutant individuals have lower fitness than the more common sensitive ones in the population in the absence of the fungicide (Skylakalis, 1987; Hollomon, 2015). Large-scale application of a new fungicide kills the sensitive individuals, but provides a selective advantage for the resistant mutants, whose fitness and population may then increase. Resistance has not been a problem with conventional non-systemic protectant fungicides, such as dithiocarbamates, phthalimides and chlorothalonil because of their multi-site modes of action that target many biochemical steps (Köller and Scheinplug, 1987; Brent, 2012; Hollomon, 2015). Developing resistance to multi-site inhibitors requires a combination of many mutations. Systemic fungicides appeared in very rapid succession in the late 1960's. Instead of forming a protective layer on the plant surface (multi-site fungicides) that could be lost through weathering, systemic inhibitors penetrate into the plants and then eradicate established infections (Brent, 2012; Hollomon, 2015). Their mode of action is typically specific and targets one biochemical site. Resistance is more common for site-specific inhibitors because mutations as small in one fungal gene are sufficient to induce a change

that can rapidly and effectively decrease binding affinity of the inhibitor within the target site (Köller and Scheinplug, 1987; Hollomon, 2015)

Since the mid-1970s, over 30 different demethylation inhibitors (DMIs) have been used in crop protection (Brent, 2012). DMIs act on only one metabolic site, C14-demethylation of lanosterol (Köller and Scheinplug, 1987). Like the other groups of systemic site-specific inhibitors, reduced sensitivity of field populations of a number of pathogens was reported in several cropping systems in the 1980s, including: barley powdery mildew (*Blumeria graminis* (DC.) Speer) (Fletcher and Wolfe, 1981; Heaney et al., 1984); cucurbit powdery mildew (*Podosphaera fusca* (Fr.) U. Braun & Shishkoff) (Huggenberger et al., 1984); barley leaf scald (*Rhynchosporium secalis* (Oudem.) Davis) (Hollomon, 1984) and; apple scab (*Venturia inaequalis* (Cooke) Wint.) (Stains and Jones, 1985; Thind et al., 1986). Repeated exposure of natural populations of target pathogens to DMIs will shift sensitivity distribution of the pathogens toward less sensitive and eventually resistant (Köller and Scheinplug, 1987; Skylakalis, 1987). In order to ensure sustained performance of fungicides against certain pathogens, disease control and resistance management strategies must be practiced. One of the often-suggested approaches is to alternate application with other fungicides that have different modes of action (Brent and Hollomon, 2007b; Brent, 2012).

In Canada, currently registered inhibitors for Monilinia blight control in wild blueberries include DMIs (propiconazole, triforine, metconazole, prothioconazole), fluazinam (uncouplers of oxidative phosphorylation), succinate dehydrogenase inhibitor (SDHI) (penthiopyrad), quinone outside inhibitor (QoI) (azoxystrobin) (combined with propiconazole as one product Quilt®), and a microbial disrupter (*Bacillus subtillis* Cohn)

(Delbridge et al., 2015). Erratic efficacy of propiconazole, triforine, metconazole, fluazinam, and *B. subtilis* has been observed in field research trials conducted to date (D. Percival, personal communication, Dalhousie University, NS, CA). Triforine has been deregistered in US and Europe and this has resulted in the inability to use this product in the production of wild blueberries that are to be potentially exported to these countries. Field research trials conducted by Percival and Beaton (2012) demonstrated that prothioconazole (active ingredient of Proline®) had the advantage of being very efficacious in managing Monilinia blight with additional benefit of no residues detected in harvested and processed berries. Prothioconazole has a triazolinthione structure and protective, curative and eradicative activity (European Food Safety Authority, 2012). *In-vitro* anti-fungal-binding studies on the pathogen *M. graminicola* CYP51 (MgCYP51) conducted by Parker et al. (2011) indicated that prothioconazole exhibited a different mechanism of inhibition from other triazoles. Prothioconazole was found to have 840-fold less binding affinity than epoxiconazole. Unlike epoxiconazole, tebuconazole, and triadimenol that are non-competitive inhibitors, prothioconazole was observed to be a competitive inhibitor of substrate binding. The anti-fungal effect of prothioconazole *in vivo* was unlikely to be attributable to the binding of prothioconazole to MgCYP51. Prothioconazole-desthio is known to be a major product of metabolism of prothioconazole in both plants and animals, and is important in the toxicology of prothioconaozle (APVMA, 2007; USEPA, 2007; JMPR, 2008). Further investigation was implemented on the human pathogen *Candida albicans* (C. P. Robin) CYP51 (CaCYP51) (Parker et al., 2013). CaCYP51 was treated with prothioconazole and prothioconazole-desthio to compare their inhibitory properties. Prothioconazole-desthio bound tightly and

non-competitively to CaCYP51 in the expected manner of triazoles. Prothioconazole, in contrast, bound weakly and competitively to CaCYP51. Prothioconazole-desthio was present in the *C. albicans* cells treated with prothioconazole. Prothioconazole-desthio was proven to be responsible for the anti-fungal activity of prothioconazole. Thus, instead of prothioconazole, prothioconazole-desthio was examined in this study. Difenoconazole, another DMI, is a promising candidate for Monilinia blight control, which is in the process of being registered. Products (Inspire Super® and Inspire®) containing difenoconazole provided relatively effective Monilinia blight control in field research trials (cf. Chapter 5).

Monilinia blight infection and Botrytis blight infection typically overlap during bloom. Applications of fungicides against Botrytis blight could have suppression effect on Monilinia blight, vice versa. SDHI (boscalid) and anilino-pyrimidine (AP) (cyprodinil) are commonly sprayed to control Botrytis blight. SDHIs inhibit the enzyme succinate dehydrogenase, also known as Complex II that resides in the mitochondrial inner membrane (the respiration chain) (Ulrich and Mathre, 1972; Keon et al., 1991). The enzyme complex SDH, consists of four subunits (A, B, C, and D), is a functional part of the tricarboxylic cycle and the mitochondrial electron transport chain and catalyzes both the reduction of quinine and the oxidation of succinate to fumarate (Keon et al., 1991; Angelini et al., 2012; Ishii, 2012). AP-fungicides inhibit methionine biosynthesis and secretion of hydrolytic enzymes (Masner et al., 1994; Miura et al., 1994).

According to the results of the propiconazole sensitivity study (cf. Chapter 3), fungicides containing propiconazole are still effective for Monilinia blight control. However, propiconazole was under the joint review of FAO/WHO and is currently under

review for re-registration in the US. This implies a high risk of it being de-registered in the near future. Available replacements and more existing effective fungicides with different modes of action will provide advantageous contributions to disease control and resistance management. Efficacy assessment of different active ingredients gives agricultural advisers and growers a guide for selecting and scheduling treatments. This laboratory experiment was conducted to investigate the efficacy of other active ingredients (prothioconazole-desthio, difenoconazole, penthiopyrad, boscalid and cyprodinil) in inhibiting the mycelial growth of *M. vaccinii-corymbosi* single-spore isolates on agar medium.

4.2 Materials and methods

A mycelium growth assay (Golembiewski et al., 1995; Hu et al., 2011; Wise et al., 2011) was used to examine the inhibiting efficacy of six technical-grade active ingredients: propiconazole (Syngenta Honeywood Research Farm, Platisville, ON, CA), prothioconazole-desthio (Bayer Crop Science, MO, US), difenoconazole (Syngenta Honeywood Research Farm, Platisville, ON, CA), penthiopyrad (DuPont, Delaware, US), boscalid (BASF Corporation-Crop Science, NC, US), and cyprodinil (Syngenta Honeywood Research Farm, Platisville, ON, CA). Ten *M. vaccinii-corymbosi* single-spore isolates were all treated with each active ingredient. These isolates were not exposed to difenoconazole, prothioconaozle-desthio, and penthiopyrad before 2014. Concentrations of propiconazole and difenoconazole: 0, 0.005, 0.01, 0.05, 0.1 $\mu g \cdot mL^{-1}$, concentrations of prothioconazole-desthio: 0, 0.001, 0.002, 0.003, 0.004 $\mu g \cdot mL^{-1}$, concentrations of penthiopyrad and boscalid: 0, 0.03, 0.3, 3, 30 $\mu g \cdot mL^{-1}$, and

concentrations of cyprodinil: 0, 5, 10, 15, 20 $\mu g \cdot mL^{-1}$ were prepared. A stock solution was prepared by dissolving active ingredients in acetone on the day of the experiment. A stock solution of each active ingredient was serially diluted to make the concentrations. The solution of each concentration of each active ingredient was added into molten potato dextrose agar (PDA) medium (approximately 50 ºC). The final concentration of acetone in PDA medium was 0.1% (v/v). Unamended medium was the control and had acetone added only. Amended PDA medium was poured into Petri dishes (90 × 15 mm) to make culture plates. Thirty plates were prepared for each isolate: six plates for each active ingredient and one plate for each concentration. Thirty mycelial plugs were removed from the margin of 7-day old colonies of each isolate using a cork borer (inner diameter: 0.4 mm), which were individually transferred and inverted onto the amended PDA plates (mycelium should be touching the medium). Procedures above were implemented in a laminar flow hood to avoid contamination. All the culture plates were labeled and sealed with parafilm, and then were incubated at 22 ± 2 °C in the dark for approximately 7 days. After a 7-day incubation, colony diameter was measured in two perpendicular directions (two perpendicular lines were previously drew at the bottom of the Petri dishes and mycelial plugs were placed at the crossing point of the two lines). The mean diameter subtracted with mycelial plug diameter (0.4 mm) was used to calculate percent relative mycelial growth of *M. vaccinii-corymbosi*.

EC_{50} analysis was conducted as previously described in Chapter 3. The experimental design was a factorial design with two fixed factors: isolate (10 levels) and active ingredient (6 levels). The whole experiment was repeated three times on different dates. Replication was the random factor. The PROC MIXED procedure of SAS (version

9.4, SAS institute, Inc., Cary, NC) was used for analysis of variance. Tukey's LSD was used for multiple means comparison. The PROC CORR procedure of SAS was used for correlation analysis.

4.3 Results and discussion

DMI fungicides are likely to be the dominant fungicide products for Monilinia blight control in wild blueberry production in Nova Scotia, because of their disease control efficiency and economic benefits. Prothioconazole-desthio and difenoconazole demonstrated a great advantage in suppressing *M. vaccinii-corymbosi* isolates. Active ingredients had significant effects on the mycelial growth of *M. vaccinii-corymbosi* on PDA at the $\alpha = 0.05$ level (Table 4-1). The EC_{50} values for cyprodinil, boscalid, penthiopyrad, propiconazole, difenoconazole and prothioconazole-desthio ranged from 7.49 to 10.07 $\mu g \cdot mL^{-1}$, 0.09 to 0.20 $\mu g \cdot mL^{-1}$, 0.016 to 0.076 $\mu g \cdot mL^{-1}$, 0.008 to 0.016 $\mu g \cdot mL^{-1}$, 0.006 to 0.0083 $\mu g \cdot mL^{-1}$and 0.00078 to 0.0012 $\mu g \cdot mL^{-1}$, respectively (Table 4-2). The mean EC_{50} values were 8.43, 0.14, 0.025, 0.012, 0.0068 and 0.00086 $\mu g \cdot mL^{-1}$, respectively. Mean EC_{50} values of each active ingredient were significantly different from one another at the level of $\alpha = 0.05$. Propiconazole, difenoconazole and prothioconazole-desthio, which have demethylation inhibiting (DMI) mode of action, were significantly more effective in inhibiting mycelial growth of *M. vaccinii-corymbosi* isolates by 50% on PDA, compared to cyprodinil, boscalid, and penthiopyrad (Table 4-2, Figure 4-1). This was similar to the observation in field research trials conducted in 2012 and 2013 (cf. Chapter 5). Prothioconazole-desthio was the most effective inhibitor that had 8-fold, 14-fold, 29-fold, 163-fold and 10^4-fold higher efficacy than difenoconazole,

propiconazole, penthiopyrad, boscalid and cyprodinil, respectively (Table 4-2). Prothioconazole-desthio and difenoconazole were always effective against each isolate, in accordance with the EC_{50} results (Table 4-3). That is, ten isolates showed consistent sensitivity to either prothioconazole-desthio or difenoconazole. Difenoconzole was more effective in inhibiting mycelial growth on PDA medium than propiconazole (Table 4-2, Figure 4-1). Propiconazole, overall, maintained its effectiveness against *M. vaccinii-corymbosi* isolates, but inconsistency existed among the isolates. Propiconazole EC_{50} value range was relatively wider than prothioconazole-desthio and difenoconazole (Table 4-2), and the EC_{50} value of isolate Farm11-19 was twice as high as the EC_{50} value of the isolate Noel13-2 (Table 4-3). The inconsistency could be attributed to its erratic efficacy, as observed in the field research trials. Since these isolates were not exposed to prothioconazole and difenoconazole before the year 2014, EC_{50} results of prothioconazole-desthio and difenoconazole provided valuable information concerning the normal variation in fungicide sensitivity of *M. vaccinii-corymbosi*, which can be used as a baseline for further monitoring sensitivity of *M. vaccinii-corymbosi* population in commercial wild blueberry fields in Nova Scotia.

Penthiopyrad could be a potential alternate to rotate with DMI fungicides. Effectiveness of penthiopyrad against *M. vaccinii-corymbosi* isolates was only 2-fold less than propiconazole. Mean EC_{50} values of penthiopyrad and propiconazole were 0.025 $\mu g \cdot mL^{-1}$ and 0.012 $\mu g \cdot mL^{-1}$, respectively. Pethiopyrad also showed inconsistent efficacy against each isolate. The EC_{50} value (0.076 $\mu g \cdot mL^{-1}$) of isolates Par13-6 was almost five times higher than the EC_{50} (0.016 $\mu g \cdot mL^{-1}$) of isolate Farm11-5 (Table 4-2, 4-3). From the perspective of reducing risk of resistance development of DMI fungicides and

increasing economic benefits, it is strategically beneficial to rotate with penthiopyrad because of its different mode of action. However, penthiopyrad was not as effective as propiconazole against *M. vaccinii-corymbosi* isolates on agar medium, it might not provide satisfactory suppression on the disease when there is high risk of *M. vaccinii-corymbosi* infection due to favorable environmental conditions such as continuous raining. Moreover, penthiopyrad also provided suppression against Botrytis blight.

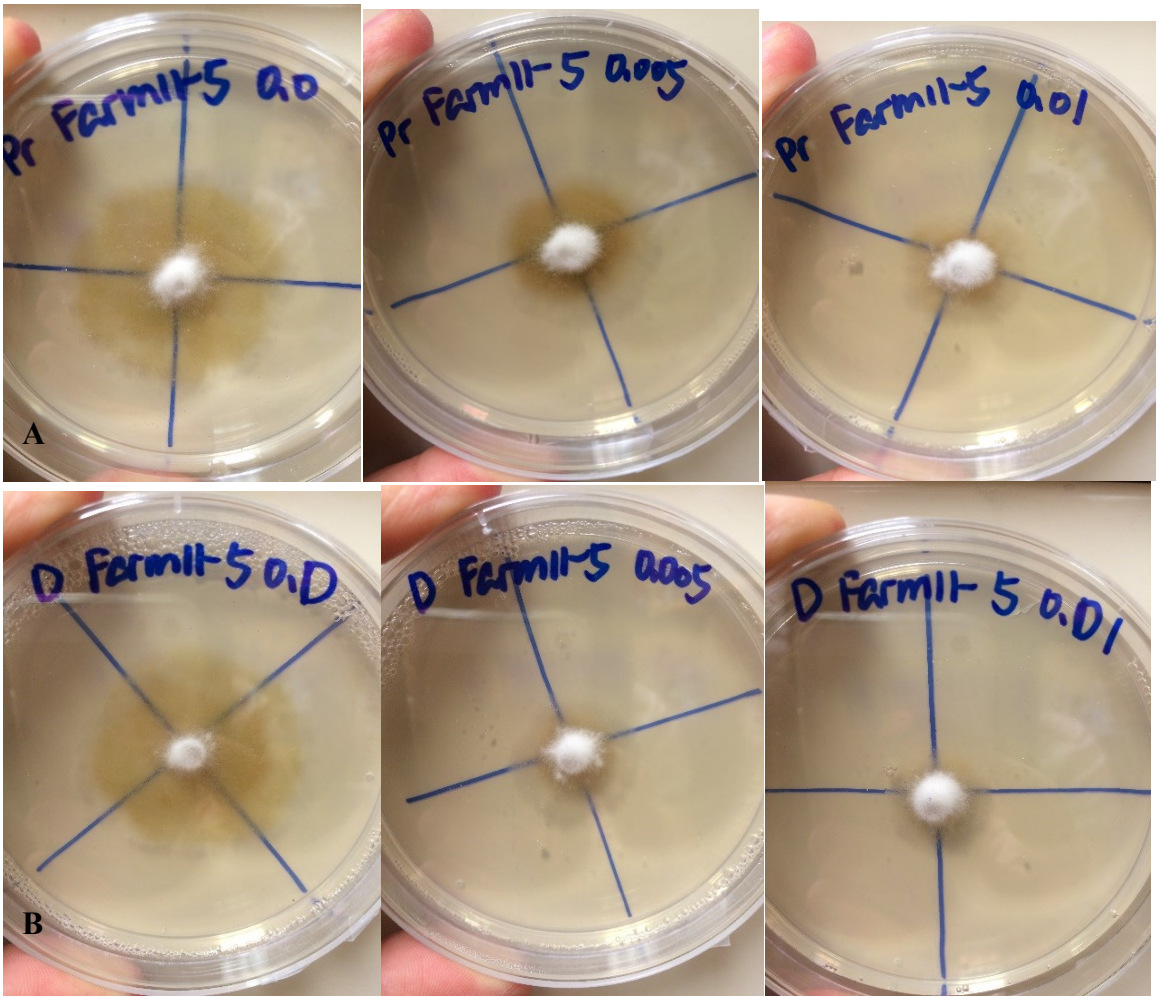


Figure 4-1 Mycelial growth of *M. vaccinii-corymbosi* isolate Farm11-5 on PDA amended with A. Propiconazole: 0, 0.005, 0.01 $\mu g \cdot mL^{-1}$; B. Difenoconazole: 0, 0.005, 0.01 $\mu g \cdot mL^{-1}$ after 7-day incubation at 22 ± 2 °C in the dark.

Table 4-1 Results of analysis of variance. Effects of ten isolates and six active ingredients (prothioconazole-desthio, propiconazole, difenoconazole, penthiopyrad, boscalid and cyprodinil), and their interaction effect

Effect	F value	*P*-value
Active ingredient	4244.47	<0.0001
Isolate	3.05	0.0027
Active ingredient*isolate	1.21	0.2119
Replication	22.02	<0.0001

Table 4-2 Inhibiting effect of each active ingredient on mycelial growth of 10 *M. vaccinii-corymbosi* single-spore isolates from commercial wild blueberry fields in Nova Scotia and their comparison using Tukey's LSD

Active ingredient	Mode of action	EC_{50} values ($\mu g \cdot mL^{-1}$)[a]		Variation factor[c]
		Range	Mean[b]	
Cyprodinil	AP	7.49-10.07	8.43 *a*	1.3
Boscalid	SDHI	0.09-0.20	0.14 *b*	2.2
Penthiopyrad	SDHI	0.016-0.076	0.025 *c*	4.8
Propiconazole	DMI	0.008-0.016	0.012 *d*	2.0
Difenoconazole	DMI	0.006-0.0083	0.0068 *e*	1.4
Prothioconazole-desthio	DMI	0.00076-0.0012	0.00086 *f*	1.6

[a] EC_{50} value is the effective concentration that inhibit mycelial growth by 50%
[b] Means that share the same letter is not significant different from each other. Proc Mixed procedure was used for analysis of variance (ANOVA)
[c] Variation factor = highest EC_{50} value divided by lowest EC_{50} value

Applications of fungicides containing boscalid or cyprodinil during bloom for Botrytis blight control are likely not to have significant impact on Monilinia blighted wild blueberry plants. Boscalid and cyprodinil were 10-fold and 700-fold less effective than propiconazole (Table 4-2), respectively. Cyprodinil was the least effective active ingredient against *M. vaccinii-corymbosi* isolates. Consistently, fungicides containing boscalid and cyprodinil have demonstrated unsatisfactory control of Monilinia blight in field research trials conducted in 2012 and 2013 (cf. Chapter 5).

Propiconazole EC_{50} values were significantly correlated with difenoconazole EC_{50} values and prothioconazole-desthio EC_{50} values at the level of α=0.05 (Table 4-4).

However, the correlation between propiconazole and difenoconazole was very weak (r = 0.39). The correlation between propiconazole and prothioconazole-desthio was much stronger (r = 0.73). Correlation implies the possible existence of cross-resistance among these DMIs. Development of reduced sensitivity or resistance of *M. vaccinii-corymbosi* to propiconazole would have impact on control efficacy of prothioconazole and difenoconazole. A significant correlation between boscalid sensitivity and penthiopyrad sensitivity was not observed among these ten *M. vaccinii-corymbosi* isolates (Table 4-5), which was in contrast to the other studies that have shown the existence of cross-resistance between boscalid and penthiopyrad (Hu et al., 2011; Amiri et al., 2014). The sample size was small in this study. A larger sample size may yield better correlation results.

Monitoring the sensitivity of the *M. vaccinii-corymbosi* population to old and new fungicides and alternate application of fungicides with different modes of action should be implemented for best management for Monilinia blight control in the wild blueberry industry. It is promising that DMIs were effective against *M. vaccinii-corymbosi* isolates *in vitro* and resistant isolates were not observed. Moreover, *M. vaccinii-corymbosi* is accepted as showing a medium risk of development of resistance to fungicides (Fungicide Resistance Action Committee, 2013b). Nevertheless, resistant mutants are assumed to exist at a low frequency (perhaps 10^{-6} to 10^{-8}) prior to the introduction of a new fungicide and the bulk of their population increase occurs before any decrease in disease control is observed, since economic disease control in the field is affected only by relatively high frequencies of resistant mutants (10^{-2} to 10^{-1}) (Skylakakis, 1987). Since prothioconazole-desthio, difenoconazole and penthiopyrad were not sprayed for Monilinia blight control

before 2014, the sensitivity results in this study provide a benchmark that can be used for future monitoring and for evaluating the development of fungicide resistance.

Table 4-3 Sensitivity of each isolate to six active ingredients cyprodinil, boscalid, penthiopyrad, propiconazole, difenoconazole, and prothioconazole-desthio

Isolate	EC_{50} values ($\mu g \cdot mL^{-1}$)[1] of each active ingredient against each isolate					
	Cyprodinil	Boscalid	Penthiopyrad	Propiconazole	Difenoconazole	Prothioconazole-desthio
Farm11-19	9.50	0.18	0.030	0.016	0.0077	0.0012
BM13-5	8.80	0.18	0.025	0.014	0.0074	0.0012
Par13-6	7.58	0.18	0.076	0.012	0.0082	0.00089
MT13-5	8.08	0.13	0.024	0.015	0.0083	0.0011
Noel13-16	10.07	0.13	0.036	0.013	0.0056	0.00094
Farm11-4	8.94	0.20	0.028	0.010	0.0062	0.00076
Farm11-5	7.49	0.15	0.016	0.013	0.0072	0.0010
Noel13-3	8.61	0.13	0.027	0.011	0.0068	0.0010
MT13-20	7.92	0.12	0.017	0.012	0.0063	0.00082
Noel13-2	7.79	0.09	0.032	0.008	0.0060	0.00077

[1] EC_{50} value is the effective concentration that inhibit mycelium growth by 50%

Table 4-4 Pearson correlation coefficient (*r*) values of EC_{50} values for propiconazole, difenoconazole, prothioconazole-desthio, boscalid and penthiopyrad

Active ingredient		DMIs				SDHI	
		Difenoconazole		Prothioconazole-desthio		Penthiopyrad	
		r	*P*-value	*r*	*P*-value	*r*	*P*-value
DMIs	Propiconazole	0.39	0.038	0.73	<0.001	0.08	0.67
	Difenoconazole	-	-	0.17	0.40	-0.17	0.41
SDHI	Boscalid	0.28	0.16	0.29	0.12	0.26	0.17

CHAPTER 5

MONILINIA BLIGHT CONTROL EFFICACY OF FUNGICIDES WITH DIFFERENT MODES OF ACTION

5.1 Introduction

Monilinia blight, also called mummy berry disease, is one of the most destructive diseases in wild and cultivated blueberry fields. The disease was first observed on wild blueberries in May, 1952, in three fields located in Kings County, Nova Scotia (Lockhart, 1961). The first survey documented 24 fields in Charlotte County, New Brunswick, of which four fields had over 50% of the blossoms and twigs infected (Lockhart, 1961). A further survey reported a complete crop loss in an 8 ha field in Antigonish County, under ideal conditions for *M. vaccinii-corymbosi* infections in 1981 (Lockhart et al., 1983).

Monilinia blight can be destructive when: i) the environmental conditions are favorable (i.e. a prolonged wetness period with conducive temperature); ii) the vegetative and floral buds of the wild blueberry plants are at susceptible stages V2 and/or F2 (V2: 2 - 5 mm green leaf tissue emerged, F2: floral bud scales separating) and; iii) ascospores have dispersed and have landed upon susceptible vegetative and/or floral buds. Once a particular field or its adjacent fields has a past history of Monilinia blight infection, this field is under high risk of Monilinia blight disease occurrence (Delbridge and Hildbrand, 1995; Annis, 2009). Intense and uniform burning, as a pruning technique, can be effective to help reduce Monilinia blight pressure, but will not sufficiently control the disease (Delbridge and Hildebrand, 1995; Lambert, 1990). In order for success in maintaining healthy crops and reliable yields of high-quality produce, fungicides are

essential for combating crop diseases, and reducing the damage they cause (Brent and Hollomon, 2007a).

In Canada, the registered fungicides for Monlinia blight control include Tilt® 250E, Mission® 418EC, and Jade® (a.i. propiconazole), Funginex® 190EC (a.i. triforine), Quash® (a.i. metconazole), Allegro® 500F (a.i. fluazinam), Fontelis® (a.i. penthiopyrad), Serenade® MAX (a.i. *Bacillus subtilis*), Proline® 480SC (a.i. prothioconazole) and Quilt® (a.i. azoxystrobin and propiconazole) (Delbridge et al., 2015). Propiconazole, triforine, metconazole and prothioconazole are demethylase inhibitors (DMIs). Propiconazole is most commonly used for Monilinia blight control of wild blueberries in Nova Scotia. Its first use occurred in 1995 (D. Percival, personal communication, Dalhousie University, NS, CA). The wild blueberry growers in Nova Scotia have been reliant on propiconazole for Monilinia blight for almost 20 years, with one to two applications being made on crop fields for Monilinia blight control. Inconsistent efficacy of propiconazole for Monilinia blight suppression has been observed in field research trials conducted in Nova Scotia in 2007, 2008, and 2010 (Percival and Beaton, 2012). Triforine, first registered for Monilinia blight control in 1978, was widely sprayed before propiconazole applications. Triforine is currently deregistered in European countries and has been re-registered only for terrestrial non-food and outdoor residential uses in the US. Blueberries sprayed with triforine products cannot be exported to the US and European countries. Moreover, triforine is no longer providing satisfactory control of Monilinia blight in wild blueberry fields in Nova Scotia (D. Percival, personal communication, Dalhousie University, NS, CA). Prothioconazole demonstrated its promising Monilinia bight control efficacy in the research trials conducted in 2009 (Percival and Beaton, 2012). Metconazole, fluazinam

and *Bacillus subtilis* have efficacy issues observed from field research trials conducted to date (D. Percival, personal communication, Dalhousie University, NS, CA).

The effectiveness of fungicides could be seriously affected by the conditions of their use, characteristics of their chemical structure and mechanisms of action of their active ingredients, and the development of resistance in target pathogens. To retain fungicide effectiveness and minimize the likelihood of fungicide resistance, it is recommended that more than two consecutive applications of the same fungicide or a fungicide that has the same mode of action (the same chemical group) be avoided (Delbridge and Rogers, 2013). Meanwhile, rotating fungicides from different chemical families or groups to lower the risk of resistance development in target pathogens is recommended. *Monilinia* species have a medium risk of development of resistance to fungicides (Fungicide Resistance Action Committee, 2013b). Occurrence of Monilinia blight in wild blueberry in Nova Scotia is widespread yearly with high disease pressure. That is, if Monilinia blight infection present, potential for spread and crop loss is high and controls must be implemented even for small populations (Agriculture and Agri-Food Canada, 2012). Since propiconazole has been extensively used for management of Monilinia blight on wild blueberry for approximately 20 years, most fungicides registered utilize a similar mode of action (DMI, demethylation inhibitors), and there are few effective fungicides with different modes of action available for rotation or alternative usage. Thus, the risk of resistance development of *M. vaccinii-corymbosi* population to propiconazole is increased. This study was conducted to investigate the ability of propiconazole, difenoconaozle, prothioconazole (DMIs) and other active ingredients with different modes of action to control Monilinia blight in commercial wild blueberry fields.

5.2 Materials and methods

5.2.1 Trial description

Field studies were conducted in 2012 and 2013 in commercial wild blueberry fields in the cropping phase of production. Two trials were located at Kemptown, Nova Scotia (NS) (coordinates = 45° 30′ N, 63° 07′ W) and Aulac, New Brunswick (NB) (coordinates = 45° 53′ N, 64° 16′ W) in 2012. The other two field trials were located at Mt. Thom, NS (coordinates = 45° 29′ N, 62° 59′ W) and Farmington, NS (coordinates = 45° 34′ N, 63° 53′ W) in 2013. Since wild blueberry fields consist of different clones, substantial variability exists within each trial. Thus, additional replications and larger sample size were used. The trials, established in April, were in a complete randomized block design with 5 replications, a plot size of 4 × 6 m, 2-m buffers between plots, and 10 treatments. Treatments included 1) untreated control (no fungicide was applied), 2) Allegro® 500F (a.i. fluazinam) applied at 2.24 L product·ha^{-1}, 3) Funginex® DC (a.i. triforine) applied at 3 product·ha^{-1}, 4) Proline® 480SC (a.i. prothioconazole) applied at 300 mL product·ha^{-1}, 5) Regalia® (a.i. extract from *Reynutria sachalinensis* (F. Schmidt) Nakai) applied at 2.8 product·ha^{-1}, 6) Pristine® WG (a.i. boscalid and pyraclostrobin) applied at 1.6 kg product·ha^{-1}, 7) Topas® 250E (a.i. propiconazole) applied at 500 mL product·ha^{-1}, 8) Inspire® (a.i. difenoconazole) applied at 80 g product·ha^{-1}, 9) Inspire Super® (a.i. cyprodinil and difenoconazole) applied at 308 g product·ha^{-1}, and 10) Vangard® 75WG (a.i. cyprodinil) applied at 180 g product·ha^{-1}. The plots were staked out and treatment tags were installed. A 2000 series Watchdog® weather station (Spectrum Technologies, Inc. Plainfield, IL, US) was set up in the centre of each trial to monitor air temperature,

relative humidity, wind speed and direction, and leaf wetness every minute. Mean values were generated every 30 min. SpecWare software (Spectrum Technologies, Inc.) was used to download the data.

5.2.2 Fungicide application

Each fungicide was mixed with distilled water and was applied using a hand-held, 2-m carbon dioxide propelled boom sprayer (Bellspray Inc. Opelousas, LA, US) with 4 nozzles and 2-L sample bottles. The nozzle type consisted of TeeJet Visiflow 8003VS (Wheaton, IL, US). The sprayer pressure was 32 PSI (220 kPa). The nozzle discharge rate was 12.5 $mL \cdot s^{-1}$ and application ground speed was approximately 1.19 $m \cdot s^{-1}$. The first application was done when the field reached to 40 – 50 % V2 and/or F2 stage (V2: 2 - 5 mm green leaf tissue emerged, F2: floral bud scales separating). The timing was decided by consulting with the *Monilinia* forecasting system (which uses temperature, wetness duration, spore dispersal, and developing stage of vegetative and/or floral buds), which was available at lowbush blueberry blog (provided by Perennia, Truro, NS, CA). The second application was done 7 to 9 days after the first application. The timing depended on the weather forecast (chance and duration of rain or fog). Infection periods were thought to have occurred prior to the second fungicide applications. In 2012, fungicide applications were made on May 2nd and May 11th at the Kemptown site and on April 30th and May 8th at the Aulac site. In 2013, fungicide applications occurred on May 8th and May 17th at the Mt. Thom site and on May 7th and May 14th at the Farmington site.

5.2.3 Disease, yield component and berry yield assessment

Fifteen blueberry stems were selected diagonally along a 4.5-m line transect in each plot, cutting the stem as close to the base as possible and avoiding vegetative stems with a pruner. Since symptoms of Monilinia bight do not show until 10 to 17 days after the susceptible buds were infected by the ascospores (Delbridge and Hildebrand, 1995), first stem collections occurred prior to the second fungicide applications, which were 7 to 9 days after first fungicide application. Second stem collections occurred within 2 weeks after the second fungicide applications. The stem samples were placed in plastic bags and taken back to the laboratory for further examination of *Monilinia* disease development, which was expressed in terms of disease incidence and disease severity. Disease incidence was determined by percentage of floral buds and/or vegetative buds per stem with visual symptoms of Monilinia blight (Figure A-4). Disease severity was determined by percentage of infected area per flower or leaf with visual symptoms of Monilinia blight within a stem (Figure A-3). Disease incidence and severity of wild blueberry plants sprayed with fungicides were compared to disease incidence and severity of wild blueberry plants not sprayed with fungicide to determine Monilinia blight suppression efficacy of the fungicides. Phytotoxicity of each fungicide was examined visually by estimating whether there was toxic damage on the floral and/or leaf buds of each stem. Physical development of each stem was determined by measuring stem length, counting number of vegetative buds and number of floral bud, and examining floral and leaf bud development stages (Figure A-1, A-2).

The impact of the treatments on yield potential was determined by examining the stems after fruit set had occurred. Stem sample collections were undertaken as described

above. The parameters examined of each stem included stem length, number of vegetative nodes, floral nodes, set fruits (premature blueberries), pinheads (no blueberry formed), and side branches.

To determine harvestable berry yield, blueberries were harvested in August with a forty-tine, commercial wild blueberry hand rake, from four randomly selected 1-m^2 quadrats in each plot. Harvested berries from each plot were weighted with an Avery Weigh Tronix® Quartzell bench scale (Avery Weigh-Tronix, LLC, Fairmont, MN, US), and the data were recorded.

5.2.4 Statistical analysis

The fixed effect was fungicide treatment with 10 levels. Replication was the random factor. Parameters of Monilinia blight disease development (disease incidence and severity), parameters of physical development of wild blueberries (stem length, number of floral and vegetative buds, development stages of floral and vegetative buds), yield components (stem length, number of vegetative nodes, floral nodes, set fruits, pinheads, and side branches), and harvested berries were examined were analyzed using the PROC MIXED procedures of SAS (version 9.3, SAS institute, Inc., Cary, NC). LSD was used for multiple means comparison at the level of $\alpha = 0.05$

5.3 Results and discussion

Monilinia blight disease pressure was low in the two trials in 2012 and the two trials in 2013, which affected the assessment of the suppressive attributes of the fungicides examined. After 2nd fungicide application, 11% and 3.5% of the total stems collected from Aulac and Kemptown respectively showed Monilinia blight symptoms (Figure 5-1) in 2012 and 28% and 7% of the total stems collected from Farmington and Mt. Thom, respectively, presented Monilinia blight symptoms in 2013. Overall, less than 0.3% of the vegetative or floral buds per stem had symptom shown in Aulac and Kemptown in 2012 (Table 5-1, 5-2). Less than 5% and 2% of vegetative or floral buds per stem had symptom shown in Farmington and Mt. Thom respectively in 2013 (Table 5-3, 5-4). Relatively dry weather (less rainfall) at the research field trials in 2012 and 2013 (Figure A-5, A-6, A-7, A-8) might contribute to low Monilinia blight disease pressure, which was contrast to severe Monilinia blight pressure in 2009 (D. Percival, personal communication, Dalhousie University, NS, CA). Each stem had 10 to 22% of the leaves or floral tissue shown visual Monilinia blight symptom in the control plots (Table 1, 2, 3) (Percival and Beaton, 2012). *M. vaccinii-corymbosi* fungus had more tendency of infecting leaf tissue than floral tissue since Monilinia blight disease incidence was higher on leaf tissues in all trials in 2012 and 2013 (Table 5-1, 5-2, 5-3, 5-4). Consistently, four trials demonstrated that all the fungicide products used in the study had no toxic effect on wild blueberry plants (Table 5-1, 5-2, 5-3, 5-4). The phytotoxicity value of each treatment in each trial was zero.

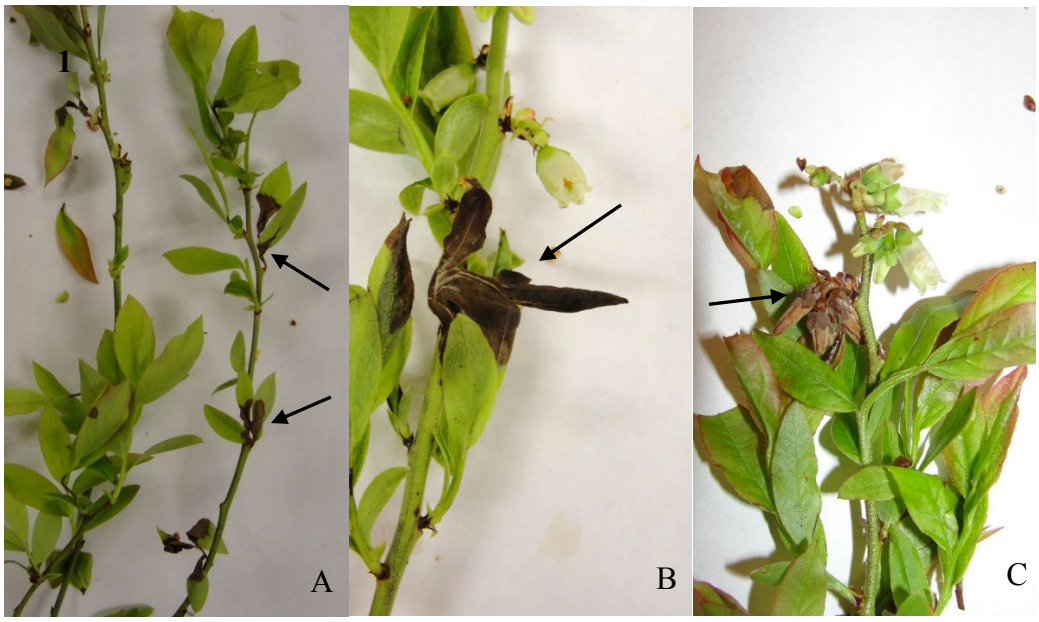


Figure 5-1 Symptoms of *M. vaccinii-corymbosi* infection on wild blueberry plants. A arrow: initial discoloration on leaf tissues; B arrow: completely discolored leaf tissues; C arrow: completely discolored floral buds

In 2012, vegetative and floral buds did not show any Monilinia blight symptoms after first fungicide application. After second fungicide application, necrotic tissues appeared on the vegetative and floral buds. At the Aulac field trial, disease incidence and severity of floral buds ranged from 0.02% to 0.25% and 0.08% to 3.13%, respectively (Table 5-1). Disease incidence and severity of vegetative buds ranged from 0.1% to 0.3% and 0.24% to 2.53%, respectively (Table 5-1). This trial did not demonstrate significant treatment effects on disease incidence or disease severity of either floral buds or vegetative buds (Table 5-1). In the Kemptown trial, floral buds were rarely infected by Monilinia blight. Disease incidence and severity of vegetative buds ranged from 0% to 0.3% and 0% to 1.47%, respectively (Table 5-2). Fungicides did not show a suppressive effect on disease incidence of vegetative buds, but slightly on disease severity of the vegetative buds (Table 5-2). Disease severity of vegetative buds treated with Proline®

480SC, Tilt® 250E, and Inspire Super® (0%, 0%, and 0% respectively) was significantly lowered by 100%.

In 2013, Monilinia blight disease pressure was higher. After the first fungicide application, only vegetative buds treated with Allegro® 500F in the Farmington trial and vegetative buds treated with Regalia in the Mt. Thom trial showed a few infected tissues. After the second fungicide application, disease incidence and severity of floral buds and vegetative buds ranged from 0% to 1.25%, 0% to 5.77%, 0.08% to 4.18%, and 2.53% to 16.3%, respectively in the Farmington trial (Table 5-3). No disease infection on floral buds in the Mt. Thom trial was observed. Disease incidence and severity of vegetative buds ranged from 0% to 1.22% and 0% to 10.4%, respectively (Table 5-4). In the Farmington trial, there was no treatment effect on disease incidence and severity of floral buds ($P > 0.05$) after the second fungicide application (Table 5-3), but there was a significant treatment effect on vegetative buds. Tilt® 250E, Proline® 480SC, and Allegro® 500F significantly lowered disease incidence of vegetative buds by 88%, 84%, and 98%, respectively (Table 5-3). Proline® 480SC and Allegro® 500F significantly lowered disease severity of vegetative buds by 75% and 84%, respectively. In the Mt. Thom trial, compared to untreated control, Funginex® DC, Allegro® 500F, Proline® 480SC, Tilt® 250E, and Inspire Super® significantly decreased disease incidence and disease severity of vegetative buds by 98% to 100% and 95% to 100%, respectively (Table 5-4).

Proline® 480SC and Tilt® 250E provided consistent control against Monilinia blight, while Allegro® 500F and Inspire Super® provided inconsistent control and Vangard® 75WG, Regalia, Pristine® WG, Inspire®, and Funginex® DC did not significantly suppress the disease in all trials. Proline® 480SC (a.i. prothioconazole),

especially, was the most effective fungicide product that reduced disease incidence of vegetative buds by 94% (Table 5-3) and 99.8% (Table 5-4) and disease severity of vegetative buds by 75% (Table 5-3), 99.5% (Table 5-4) and 100% (Table 5-2), which was followed by Tilt® 250E (a.i. propiconazole) which reduced disease incidence of vegetative buds by 88% (Table 5-3), 99.8% (Table 5-4) and disease severity of vegetative buds by 54% (Table 5-3), 99.7% (Table 5-4) and 100% (Table 5-2). These are similar to the results observed in field research trials conducted in 2009 (Percival and Beaton, 2012). Prothioconazole proved to be the most effective active ingredient inhibiting mycelial growth of *M. vaccinii-corymbosi* isolates on amended agar medium (cf. Chapter 4). Propiconazole was less effective than prothioconazole, but it was still effective against *M. vaccinii-corymbosi* isolates on amended agar medium. Meanwhile, resistance or reduced sensitivity of *M. vaccinii-corymbosi* isolates to propiconazole was not observed *in vitro* (cf. Chapter 3). The *in-vitro* results was consistent with the *in-vivo* results that Proline® 480SC and Tilt® 250E had higher control efficacy against Monilinia blight in the field trials. Difenoconazole, the active ingredient of Inspire®, was effective in inhibiting the mycelial growth of *M. vaccinii-corymbosi* isolates *in vitro* (cf. Chapter 4). Inspire Super® contained difenoconazole and cyprodinil had 100% reduction of visual symptoms of Monilinia blight on vegetative buds in the Kemptown trial in 2012 and the Mt. Thom trial in 2013, but did not suppress the disease significantly in the Farmington trial in 2013. Cyprodinil did not effectively inhibit mycelial growth of *M. vaccinii-corymbosi* isolates *in vitro*, which might be attributed to the inconsistent efficacy of Inspire Super® *in vivo*. Vangard® contained cyprodinil also did not suppress Monilnia blight in the field

trial. However, it is noted that Inspire® (a.i. difenoconazole) did not suppress Monilinia blight in the field trials.

In 2012 and 2013, there was no treatment effect on physical development of wild blueberry plants after the first and second fungicide applications in all trials (Table 5-5, 5-6, 5-7, and 5-8). Parameters of physical development included stem length, number of floral and/or vegetative buds per stem, development stages of floral and/or vegetative buds. Wild blueberry plants of each treatment in each trial did not have significantly different yield components (include stem length, number of vegetative nodes and/or floral nodes per stem, number of pinhead, set fruit and side branches per stem) before harvesting, nor were berry yields significantly different among treatments (Table 5-9, 5-10, 5-11, and 5-12). Merely a few floral buds or none were infected by *M. vaccinii-corymbosi* in the field trials, and the disease pressure on the vegetative buds was low as well, which led to no significant impact on berry yield.

Fungicide products containing demethylation inhibitors (DMIs) were the most effective against Monilinia blight control in the field research trials; especially Proline® 480SC which contained the active ingredient prothioconazole provided significant Monilinia control in three trials (Kemptown, Farmington, and Mt. Thom). Tilt® 250E (a.i. propiconazole) also provided significant Monilinia blight control in these three trials. Nevertheless, fungicides containing difenoconazole (Inspire® and Inspire Super®) did not demonstrate satisfactory Monilinia blight control in all field research trials. Inspire Super® provided complete disease control in the Kemptown and Mt. Thom trials, but Inspire® did not significantly control the disease. Besides difenoconazole, *in-vivo* Monilinia blight control efficacy of prothioconazole and propiconazole was consistent

with *in-vitro* effectiveness in inhibiting mycelial growth of *M. vaccinii-corymbosi* on agar medium.

Table 5-1 Influence of nine fungicides with varying modes of action on the suppression of Monilinia blight of wild blueberries at a commercial field located in Aulac, New Brunswick during the 2012 growing season

	Treatment	After 1st fungicide application					After 2nd fungicide application				
		Disease incidence[a] (%)		Disease severity[b] (%)		Phyto-toxicity (Yes=1, No=0)	Disease incidence (%)		Disease severity (%)		Phyto-toxicity (Yes=1, No=0)
		FB[c]	VB[d]	FB	VB		FB	VB	FB	VB	
1	Untreated control	0.00	0.00	0.00	0.00	0	0.09	0.28	0.68	2.23	0
2	Allegro® 500F	0.00	0.00	0.00	0.00	0	0.11	0.22	0.99	2.19	0
3	Funginex® DC	0.00	0.00	0.00	0.00	0	0.06	0.10	0.26	0.24	0
4	Proline® 480SC	0.00	0.00	0.00	0.00	0	0.11	0.12	0.57	0.40	0
5	Regalia	0.00	0.00	0.00	0.00	0	0.22	0.30	3.13	2.53	0
6	Pristine® WG	0.00	0.00	0.00	0.00	0	0.24	0.21	2.88	0.95	0
7	Tilt® 250E	0.00	0.00	0.00	0.00	0	0.13	0.16	0.43	0.48	0
8	Inspire®	0.00	0.00	0.00	0.00	0	0.25	0.24	1.91	0.87	0
9	Inspire Super®	0.00	0.00	0.00	0.00	0	0.02	0.19	0.08	0.67	0
10	Vangard® 75WG	0.00	0.00	0.00	0.00	0	0.06	0.22	0.20	0.95	0
ANOVA results[e]							NS	NS	NS	NS	

[a] Disease incidence = 0 to 100% where 0 = no floral/vegetative buds affected and 100 = all floral/vegetative buds are affected with at least one lesion.

[b] Disease severity = 0 to 100% where 0 = no disease and 100 = one whole floral/vegetative bud is affected.

[c,d] FB = floral bud; VB = vegetative bud

[e] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\acute{\alpha}=0.05$). Values share the same letter are not significantly different from each other.

Table 5-2 Influence of nine fungicides with varying modes of action on the suppression of Monilinia blight of wild blueberries at a commercial field located in Kemptown, Nova Scotia during the 2012 growing season

	Treatment	After 1st fungicide application					After 2nd fungicide application				
		Disease incidence[a] (%)		Disease severity[b] (%)		Phyto-toxicity (Yes=1, No=0)	Disease incidence (%)		Disease severity (%)		Phyto-toxicity (Yes=1, No=0)
		FB[c]	VB[d]	FB	VB		FB	VB	FB	VB	
1	Untreated control	0.00	0.00	0.00	0.00	0	0.00	0.30	0.00	1.01 *ab*	0
2	Allegro® 500F	0.00	0.00	0.00	0.00	0	0.00	0.26	0.00	1.47 *a*	0
3	Funginex® DC	0.00	0.00	0.00	0.00	0	0.00	0.04	0.00	0.16 *bc*	0
4	Proline® 480SC	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00 *c*	0
5	Regalia	0.00	0.00	0.00	0.00	0	0.07	0.24	1.20	0.88 *abc*	0
6	Pristine® WG	0.00	0.00	0.00	0.00	0	0.00	0.16	0.00	0.40 *abc*	0
7	Tilt® 250E	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00 *c*	0
8	Inspire®	0.00	0.00	0.00	0.00	0	0.06	0.07	1.00	0.23 *bc*	0
9	Inspire Super®	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00 *c*	0
10	Vangard® 75WG	0.00	0.00	0.00	0.00	0	0.15	0.22	2.10	1.27 *ab*	0
ANOVA results[e]								NS		*P*=0.04	

[a] Disease incidence = 0 to 100% where 0 = no floral/vegetative buds affected and 100 = all floral/vegetative buds are affected with at least one lesion.

[b] Disease severity = 0 to 100% where 0 = no disease and 100 = one whole floral/vegetative bud is affected.

[c,d] FB = floral bud; VB = vegetative bud

[e] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\acute{\alpha}=0.05$). Values share the same letter are not significantly different from each other.

Table 5-3 Influence of nine fungicides with varying modes of action on the suppression of Monilinia blight of wild blueberries at a commercial field located in Farmington, Nova Scotia during the 2013 growing season

	Treatment	After 1st fungicide application					After 2nd fungicide application				
		Disease incidence[a] (%)		Disease severity[b] (%)		Phyto-toxicity (Yes=1, No=0)	Disease incidence (%)		Disease severity (%)		Phyto-toxicity (Yes=1, No=0)
		FB[c]	VB[d]	FB	VB		FB	VB	FB	VB	
1	Untreated control	0.00	0.00	0.00	0.00	0	0.45	4.18 *a*	2.28	15.4 *ab*	0
2	Allegro® 500F	0.00	0.13	0.00	0.07	0	0.00	0.08 *d*	0.00	2.53 *d*	0
3	Funginex® DC	0.00	0.00	0.00	0.00	0	0.29	1.10 *abcd*	1.79	9.73 *abcd*	0
4	Proline® 480SC	0.00	0.00	0.00	0.00	0	0.46	0.24 *cd*	2.56	3.87 *cd*	0
5	Regalia	0.00	0.00	0.00	0.00	0	1.25	3.83 *a*	5.77	18.0 *a*	0
6	Pristine® WG	0.00	0.00	0.00	0.00	0	0.00	0.93 *abcd*	0.00	6.53 *bcd*	0
7	Tilt® 250E	0.00	0.00	0.00	0.00	0	0.27	0.51 *bcd*	1.50	7.33 *abcd*	0
8	Inspire®	0.00	0.00	0.00	0.00	0	0.36	3.07 *ab*	1.91	13.8 *abc*	0
9	Inspire Super®	0.00	0.00	0.00	0.00	0	0.47	3.64 *a*	2.49	15.6 *ab*	0
10	Vangard® 75WG	0.00	0.00	0.00	0.00	0	0.22	2.41 *abc*	1.97	16.3 *ab*	0
ANOVA results[e]							NS	*P*=0.02	NS	*P*=0.048	

[a] Disease incidence = 0 to 100% where 0 = no floral/vegetative buds affected and 100 = all floral/vegetative buds are affected with at least one lesion.

[b] Disease severity = 0 to 100% where 0 = no disease and 100 = one whole floral/vegetative bud is affected.

[c,d] FB = floral bud; VB = vegetative bud

[e] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\acute{\alpha}=0.05$). Values share the same letter are not significantly different from each other.

Table 5-4 Influence of nine fungicides with varying modes of action on the suppression of Monilinia blight of wild blueberries at a commercial field located in Mt. Thom, Nova Scotia during the 2013 growing season

	Treatment	After 1st fungicide application					After 2nd fungicide application				
		Disease incidence[a] (%)		Disease severity[b] (%)		Phyto-toxicity (Yes=1, No=0)	Disease incidence (%)		Disease severity (%)		Phyto-toxicity (Yes=1, No=0)
		FB[c]	VB[d]	FB	VB		FB	VB	FB	VB	
1	Untreated control	0.00	0.00	0.00	0.00	0	0.00	1.220 *a*	0.00	6.18 *ab*	0
2	Allegro® 500F	0.00	0.00	0.00	0.00	0	0.00	0.016 *bc*	0.00	0.09 *cd*	0
3	Funginex® DC	0.00	0.00	0.00	0.00	0	0.00	0.020 *bc*	0.00	0.28 *bcd*	0
4	Proline® 480SC	0.00	0.00	0.00	0.00	0	0.00	0.003 *bc*	0.00	0.03 *cd*	0
5	Regalia	0.00	0.19	0.00	1.2	0	0.00	0.220 *ab*	0.00	1.85 *abc*	0
6	Pristine® WG	0.00	0.00	0.00	0.00	0	0.00	0.160 *abc*	0.00	0.99 *abcd*	0
7	Tilt® 250E	0.00	0.00	0.00	0.00	0	0.00	0.003 *bc*	0.00	0.02 *cd*	0
8	Inspire®	0.00	0.00	0.00	0.00	0	0.00	0.140 *abc*	0.00	0.89 *abcd*	0
9	Inspire Super®	0.00	0.00	0.00	0.00	0	0.00	0.000 *c*	0.00	0.00 *d*	0
10	Vangard® 75WG	0.00	0.00	0.00	0.00	0	0.00	1.050 *a*	0.00	10.40 *a*	0
ANOVA results[e]								*P*=0.01		*P*=0.02	

[a] Disease incidence = 0 to 100% where 0 = no floral/vegetative buds affected and 100 = all floral/vegetative buds are affected with at least one lesion.

[b] Disease severity = 0 to 100% where 0 = no disease and 100 = one whole floral/vegetative bud is affected.

[c,d] FB = floral bud; VB = vegetative bud

[e] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\acute{\alpha}=0.05$). Values share the same letter are not significantly different from each other.

Table 5-5 Influence of nine fungicides with varying modes of action on the physical development of wild blueberries at a commercial field located in Aulac, New Brunswick during the 2012 growing season

Treatment		After 1st fungicide application					After 2nd fungicide application				
		Stem length (cm)	# of FB[a]	# of VB[b]	FB stage	VB stage	Stem length (cm)	# of FB	# of VB	FB stage	VB stage
1	Untreated control	16.6	15.2	11.6	3	4	15.7	10.3	13.2	3	4
2	Allegro® 500F	15.8	11.8	13.4	2	3	16.1	9.5	12.7	3	4
3	Funginex® DC	14.6	11.7	10.7	3	3	14.1	10.5	11.9	3	4
4	Proline® 480SC	15.4	12.8	11.3	3	3	16.3	9.3	11.3	3	4
5	Regalia	15.7	12.2	11.8	3	3	16.3	9.3	12.6	3	4
6	Pristine® WG	16.5	14.8	11.2	3	3	15.6	13.8	11.6	3	4
7	Tilt® 250E	16.4	13.7	11.4	3	3	15.5	8.2	10.9	3	4
8	Inspire®	15.7	12.1	13.0	3	4	16.6	8.3	13.4	3	4
9	Inspire Super®	15.4	13.9	13.0	3	4	16.3	9.6	12.5	3	4
10	Vangard® 75WG	16.5	11.7	12.6	3	3	16.6	8.9	12.8	3	4
ANOVA results[c]		NS	NS	NS			NS	NS	NS		

[a,b] FB = floral bud; VB = vegetative bud

[c] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\alpha=0.05$). Values share the same letter are not significantly different from each other.

Table 5-6 Influence of nine fungicides with varying modes of action on the physical development of wild blueberries at a commercial field located in Kemptown, Nova Scotia during the 2012 growing season

Treatment		After 1st fungicide application					After 2nd fungicide application				
		Stem length (cm)	# of FB[a]	# of VB[b]	FB stage	VB stage	Stem length (cm)	# of FB	# of VB	FB stage	VB stage
1	Untreated control	21.0	9.3	11.6	2	2	19.5	7.3	14.7	3	4
2	Allegro® 500F	22.4	8.8	11.7	2	2	21.2	9.4	12.4	3	4
3	Funginex® DC	22.9	10.3	11.8	2	2	23.1	7.8	13.0	3	4
4	Proline® 480SC	23.2	8.1	12.4	2	2	24.0	7.9	14.6	3	4
5	Regalia	21.5	10.7	12.2	2	2	22.4	9.8	14.3	3	4
6	Pristine® WG	21.8	8.8	10.5	2	2	23.6	9.3	14.4	3	4
7	Tilt® 250E	22.4	11.9	13.4	2	2	21.3	9.3	11.9	3	4
8	Inspire®	23.2	12.2	10.2	2	2	21.9	8.5	12.3	3	4
9	Inspire Super®	23.7	8.4	12.1	1	2	23.8	6.2	15.1	2	4
10	Vangard® 75WG	21.1	10.0	10.9	2	2	21.9	8.9	13.8	3	4
ANOVA results[c]		NS	NS	NS			NS	NS	NS		

[a,b] FB = floral bud; VB = vegetative bud

[c] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\alpha=0.05$). Values share the same letter are not significantly different from each other.

Table 5-7 Influence of nine fungicides with varying modes of action on the physical development of wild blueberries at a commercial field located in Farmington, Nova Scotia during the 2013 growing season

Treatment		After 1st fungicide application					After 2nd fungicide application				
		Stem length (cm)	# of FB[a]	# of VB[b]	FB stage	VB stage	Stem length (cm)	# of FB	# of VB	FB stage	VB stage
1	Untreated control	21.4	5.9	14.5	3	4	22.7	9.7	17.9	> 3	> 4
2	Allegro® 500F	23.3	9.0	18.0	3	4	21.1	9.4	15.9	> 3	> 4
3	Funginex® DC	25.5	9.1	18.7	3	4	23.0	6.6	15.3	> 3	> 4
4	Proline® 480SC	22.9	10.0	18.2	3	4	21.1	7.4	17.3	> 3	> 4
5	Regalia	21.8	7.2	14.7	3	4	21.8	8.1	15.6	> 3	> 4
6	Pristine® WG	21.2	6.3	13.7	3	4	22.1	7.7	15.6	> 3	> 4
7	Tilt® 250E	21.8	8.2	17.2	3	4	19.5	7.2	14.8	> 3	> 4
8	Inspire®	20.7	7.6	16.7	3	4	20.2	9.1	16.9	> 3	> 4
9	Inspire Super®	22.5	8.6	15.4	3	4	20.7	7.5	15.7	> 3	> 4
10	Vangard® 75WG	22.8	6.8	17.1	3	4	24.0	7.8	16.0	> 3	> 4
ANOVA results[c]		NS	NS	NS			NS	NS	NS		

[a,b] FB = floral bud; VB = vegetative bud

[c] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\alpha=0.05$). Values share the same letter are not significantly different from each other.

Table 5-8 Influence of nine fungicides with varying modes of action on the physical development of wild blueberries at a commercial field located in Mt. Thom, Nova Scotia during the 2013 growing season

Treatment		After 1st fungicide application					After 2nd fungicide application				
		Stem length (cm)	# of FB[a]	# of VB[b]	FB stage	VB stage	Stem length (cm)	# of FB	# of VB	FB stage	VB stage
1	Untreated control	20.7	5.5	15.3	3	4	20.5	5.2	14.2	> 3	> 4
2	Allegro® 500F	22.3	4.3	17.0	3	4	21.3	4.4	18.2	> 3	> 4
3	Funginex® DC	23.3	4.6	15.0	3	4	20.3	3.8	15.8	> 3	> 4
4	Proline® 480SC	21.0	5.7	15.3	3	4	20.8	6.1	15.4	> 3	> 4
5	Regalia	19.9	5.0	17.8	3	4	18.8	4.4	15.4	> 3	> 4
6	Pristine® WG	21.7	4.5	16.8	3	4	20.2	3.8	15.1	> 3	> 4
7	Tilt® 250E	19.3	5.8	13.5	3	4	18.6	3.8	12.6	> 3	> 4
8	Inspire®	21.7	4.5	16.1	3	4	20.1	4.1	16.8	> 3	> 4
9	Inspire Super®	20.7	5.1	16.4	3	4	19.7	4.9	16.5	> 3	> 4
10	Vangard® 75WG	20.4	5.0	18.7	3	4	21.3	4.6	16.2	> 3	> 4
ANOVA results[c]		NS	NS	NS			NS	NS	NS		

[a,b] FB = floral bud; VB = vegetative bud

[c] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\alpha=0.05$). Values share the same letter are not significantly different from each other.

Table 5-9 Influence of nine fungicides with varying modes of action on the yield components and harvested berry yield of wild blueberries at a commercial field located in Aulac, New Brunswick during the 2012 growing season

Treatment		Stem length (cm)	No. of vegetative nodes	No. of floral nodes	No. of pinhead	No. of set fruit	No. of side branches	Berry yield ($g \cdot m^{-2}$)
1	Untreated control	12.8	9.2	5.9	3.9	18.9	1.6	623.2
2	Allegro® 500F	14.7	11	6.5	2.4	22.5	1.7	765.7
3	Funginex® DC	13.5	10.1	5.3	2.4	17.8	1.7	460.0
4	Proline® 480SC	14.4	9.5	6.1	2.6	23.2	1.6	794.9
5	Regalia	13.6	8.4	6.8	2.2	22.7	1.6	525.1
6	Pristine® WG	15.4	9.8	6.7	3.3	24.9	1.6	798.1
7	Tilt® 250E	15.5	10.3	6.2	2.3	27.3	1.6	758.4
8	Inspire®	16.2	10.0	5.7	1.9	23.5	1.3	752.2
9	Inspire Super®	14.4	10.3	6.2	2.7	21.4	1.6	629.3
10	Vangard® 75WG	14.6	9.6	4.9	1.2	22.5	1.2	785.5
ANOVA results[a]		NS	NS	NS	NS	NS	NS	NS

[a] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\acute{\alpha}=0.05$). Values share the same letter are not significantly different from each other.

Table 5-10 Influence of nine fungicides with varying modes of action on the yield components and harvested berry yield of wild blueberries at a commercial field located in Kemptown, Nova Scotia during the 2012 growing season

Treatment		Stem length (cm)	No. of vegetative nodes	No. of floral nodes	No. of pinhead	No. of set fruit	No. of side branches	Berry yield ($g \cdot m^{-2}$)
1	Untreated control	19.5	12.7	5.6	3.9	22.0	1.5	533.1
2	Allegro® 500F	22.1	14.0	4.9	4.1	17.5	1.3	587.0
3	Funginex® DC	22.7	12.5	6.2	3.9	19.4	1.2	593.7
4	Proline® 480SC	21.2	14.5	4.5	3.2	13.0	1.7	509.5
5	Regalia	21.1	13.5	6.3	4.6	21.0	1.8	477.2
6	Pristine® WG	21.4	13.3	1.6	4.9	13.8	1.0	526.2
7	Tilt® 250E	20.5	12.9	6.3	2.7	22.0	1.4	523.9
8	Inspire®	23.1	15.6	7.4	4.1	28.1	1.7	683.4
9	Inspire Super®	22.8	15.5	4.2	4.0	11.9	1.6	425.3
10	Vangard® 75WG	22.6	13.3	6.6	4.6	17.5	2.5	427.9
ANOVA results[a]		NS	NS	NS	NS	NS	NS	NS

[a] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\acute{\alpha}=0.05$). Values share the same letter are not significantly different from each other.

Table 5-11 Influence of nine fungicides with varying modes of action on the yield components and harvested berry yield of wild blueberries at a commercial field located in Farmington, Nova Scotia during the 2013 growing season

Treatment		Stem length (cm)	No. of vegetative nodes	No. of floral nodes	No. of pinhead	No. of set fruit	No. of side branches	Berry yield ($g \cdot m^{-2}$)
1	Untreated control	19.8	11.7	5.9	1.3	15.1	1.0	540.4
2	Allegro® 500F	19.9	13.7	6.2	1.8	20.7	1.3	625.7
3	Funginex® DC	19.8	13.5	6.3	2.2	17.4	1.3	525.2
4	Proline® 480SC	21.4	12.9	5.6	1.3	15.8	1.0	762.6
5	Regalia	20.0	10.3	5.3	0.9	13.4	0.8	543
6	Pristine® WG	19.3	13.3	6.1	1.9	19.8	0.9	781.4
7	Tilt® 250E	18.7	9.7	5.0	0.8	16.0	0.5	825.8
8	Inspire®	20.0	9.8	5.3	1.2	14.0	1.4	651.6
9	Inspire Super®	20.3	11.2	6.3	0.6	17.2	1.5	716.8
10	Vangard® 75WG	21.6	10.9	4.8	1.0	14.2	1.0	486.1
ANOVA results[a]		NS	NS	NS	NS	NS	NS	NS

[a] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\acute{\alpha}=0.05$). Values share the same letter are not significantly different from each other.

Table 5-12 Influence of nine fungicides with varying modes of action on the yield components and harvested berry yield of wild blueberries at a commercial field located in Mt. Thom, Nova Scotia during the 2013 growing season

Treatment		Stem length (cm)	No. of vegetative nodes	No. of floral nodes	No. of pinhead	No. of set fruit	No. of side branches	Berry yield ($g \cdot m^{-2}$)
1	Untreated control	19.7	10.0	4.2	0.1	14.4	1.3	361.3
2	Allegro® 500F	18.5	13.9	5.1	0.4	17.5	1.3	386.2
3	Funginex® DC	18.2	12.9	4.3	0.1	14.5	1.5	458.1
4	Proline® 480SC	18	13.2	5.0	0.6	17.2	1.6	410.0
5	Regalia	19.2	13.5	3.7	0.4	12.0	1.4	302.9
6	Pristine® WG	18.3	14.0	6.3	0.9	22.6	1.3	536.9
7	Tilt® 250E	16.5	10.5	3.9	0.4	11.8	1.0	413.0
8	Inspire®	19.6	14.4	4.3	0.9	15.2	0.9	506.5
9	Inspire Super®	18	13.5	4.2	0.3	14.4	1.9	424.7
10	Vangard® 75WG	20.6	12.4	4.3	0.08	13.5	1.6	298.8
ANOVA results[a]		NS	NS	NS	NS	NS	NS	NS

[a] Analysis of variance (ANOVA) results refer to treatment effects that were either not significant (NS) or significant at $p<0.05$. Mean separation was completed using LSD multiple means comparison test procedure ($\acute{\alpha}=0.05$). Values share the same letter are not significantly different from each oth

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

One objective of the study was to examine whether the *M. vaccinii-corymbosi* population in the commercial wild blueberry fields in Nova Scotia has developed reduced sensitivity or resistance under the selection pressure of active ingredient propiconazole which has been used for approximately 20 years. One hundred and two single-spore isolates were tested *in vitro* using mycelial growth assay. The results indicated that the *M. vaccinii-corymbosi* population had not developed reduced sensitivity to propiconazole. Propiconazole resistant isolates were also not detected.

The other objective was to investigate the efficacy of fungicides with varying modes of action against *M. vaccinii-corymbosi in vitro* and *in vivo*. *In vitro*, 10 of the 102 single-spore isolates were selected to investigate mycelial inhibiting effectiveness of demethylation inhibitors (DMIs: prothiconazole, difenoconazole, and propiconazole), succinate dehydrogenase inhibitors (SDHIs: penthiopyrad and boscalid), and anilino-pyrimidine (AP: cyprodinil). DMIs were illustrated to be the most effective inhibitors, suppressing mycelial growth of *M. vaccinii-corymbosi* isolates on PDA medium, which was followed by SDHIs. Cyprodinil was the least effective inhibitor. According to EC_{50} results, prothioconazole and difenoconazole may be advantageous candidates to replace propiconazole, and penthiopyrad, as beneficial alternatives to rotate with propiconazole, to avoid extensive usage of fungicide products with the same mode of action and reduce risk of resistance development. *In vivo*, wild blueberry plants were treated with nine

fungicides with varying modes of action. DMI fungicides were the most consistent and effective products for Monilinia blight control in field research trials. Three of four trials had significant treatment effect on disease incidence and/or disease severity of vegetative buds and/or floral buds, where prothioconazole (fungicide: Proline® 480SC) and propiconazole (fungicide: Topas® 250E) had significant suppression on *M. vaccinii-corymbosi* infection. Nevertheless, difenoconazole (fungicide: Inspire® and Inspire Super®) provided inconsistent control of Monilinia blight in the field. Inspire® did not significantly suppress Monilinia blight in these three trials and Inspire Super® had significant Monilinia blight control in two trials, but not in the other trial. Moreover, cyprodinil (fungicide: Vangard® 75WG) was also not effective for Monilinia blight control *in vivo*.

M. vaccinii-corymbosi population in commercial wild blueberry fields in NS is still sensitive to DMIs (prothioconazole, difenoconazole, and propiconazole), and the selection pressure of propiconazole after 20-year exposure is maintained at a low level. No isolates were detected to have resistance to propiconazole. The results are consistent with the pathogen risk classification by Fungicide Resistance Action Committee (2013b) (CropLife International, Brussels, Belgium), where Monilinia species are accepted as showing a medium risk of developing to fungicides (resistance of the pathogen has not created major problem or has been slow to develop in commercial practice). To date, Thompson and Annis (2014) have reported reduced sensitivity of *M. vaccinii-corymbosi* population to propiconazole in conventionally managed wild blueberry fields in Maine, US but resistant isolates were not detected.

6.2 Recommendations

According to the results from the *in-vitro* sensitivity study of *M. vaccinii-corymbosi* isolates to different active ingredients and the *in-vivo* fungicide efficacy examination for Monilinia blight control, *M. vaccinii-corymbosi* population had no reduced sensitivity or resistance developed to propiconazole, DMIs were the most effective class of fungicides. However, propiconazole was under the joint review by the FAO/WHO and is currently under review by EPA in the US. This implies a high risk of being de-registered in the near future. It is beneficial to have alternatives available. Proline® 480SC (a.i. prothioconazole) is a promising candidate to replace propiconazole-containing fungicides, such as Tilt® 250E. Inspire® (a.i. difenoconazole) and Inspire Super® (difenoconazole and cyprodinil) are in the process of registration. According to the *in-vitro* sensitivity results of difenoconazole, Inspire® can be another potential candidate. Cyprodinil was not effective in suppressing *M. vaccinii-corymbosi in vitro* or *in vivo*, which may influence the effectiveness of Inspire Super® and its registration for Monilinia blight control. In the perspective of resistance management, it is important to rotate applications with fungicides that have different modes of action, since repeated usage of one class of fungicide such as DMI, will increase the risk of resistance development. *In-vitro* effectiveness of penthiopyrad against *M. vaccinii-corymbosi* was 2-fold less than propiconazole. It is strategically beneficial to rotate usage with penthiopyrad that has different mode of action from DMI under the conditions of low to moderate risk of *M. vaccinii-corymbosi* infection. Furthermore, since isolates obtained in the study have no exposure history to prothioconazole, difenoconazole and penthiopyrad, sensitivity of the isolates to each of the three active ingredients or a new fungicide

candidate is considered to be a baseline, which can be used to determine whether reduced sensitivity is occurring in future monitoring and evaluation.

Infection by *M. vaccinii-corymbosi* (Monilinia blight) in wild blueberry plants occurs when i) vegetative and floral buds develop to susceptible stage of V2 and/or F2 (V2: 2 - 5 mm green tissue emerged, F2: bud scales separating), ii) ascospores discharged from apothecia land on the susceptible buds, and iii) the environmental conditions are favorable (i.e. prolonged wetness period with conducive temperature). Milholland (1977) observed that germinated ascospores of *M. vaccinii-coyrmbosi* directly penetrated into an epidermal cell and indirectly penetrated through a stomata after 48 hours after inoculating ascospores on the highbush blueberry leaves at 20 - 25 ºC. Thus, to protect wild blueberry plants from Monilinia blight disease, fungicides should be applied before ascospore infection occurs, which is within 48 hours after onset of wetness duration. It is more advantageous to protect the plants from pathogen infection, rather than to cure diseased plants. According to the life cycle of *M. vaccinii-corymbosi*, secondary infection (mummification of berries) occurs by asexual spores (conidia) produced from blighted plant tissues, resulting from primary infection by sexual spores (ascospores). If primary infection control is carried out, there would be no concern for secondary infection.

Wild blueberry fields have genetically diverse clones, each of which react differently to *M. vaccinii-corymbosi* infection, due to their unique phenotypes. For instance, hairy leaves and stems of *V. myrtilloides* provide inherent resistance to *M. vaccinii-corymbosi*, and late breaking buds of *V. myrtilloides* provide avoidance of *M. vaccinii-corymbosi* ascospore infection during sporulation. By contrast, *V. angustifolium* f. *nigrum* (Wood) lacks hair on the mid-vein of the leaf. *V. angustifolium* f. *nigrum* has

been reported to have more severe Monilinia bight infection than the other clones in the wild blueberry fields (Lockhart et al., 1983). Different phenotypes of wild blueberry plants in the field research trials may have influence on the Monilinia blight control efficacy of the fungicides. Further investigation on different phenotypes of wild blueberry plants and their responses to *M. vaccinii-corymbosi* infection would be beneficial for Monilinia blight disease management.

Fungus isolated from Monilinia-blighted tissues of wild blueberry plants and mummy berries was identified as *M. vaccinii-corymbosi*, according to their morphological characteristics and identification results from the National Fungal Identification Service Gestionnaire (NFISG) (Agriculture and Agri-Food Canada, Ottawa, ON, CA) (unpublished data). However, the ITS sequencing result obtained from the NFISG lab was different from the ITS sequencing result from the study by Holst-Jensen et al. (1997). Further investigation is inquired.

REFERENCES

Agriculture and Agri-Food Canada. 2012. Crop profile for lowbush blueberry in Canada, 2011.[Online] Available: http://publications.gc.ca/collections/collection_2012/agr/A118-10-31-2012-eng.pdf [May 15, 2015].

Ambrus, Á, Velde-Koerts, T. and Ossendorp, B.C. 1987. Propiconazole. The Joint FAO/WHO Meeting on Pesticide Residues, [Online] Available:http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation07/Propiconazole.pdf [Jun 08, 2015]

Amiri, A., Heath, S.M. and Peres, N.A. 2014. Resistance to fluopyram, fluxapyroxad, and penthiopyrad in Botrytis cinerea from strawberry. Plant Disease, 98: 532-539

Angelini, R.M.D.M., Pollastro, S. and Faretra, F. 2012. Genetics of fungicide resistance in *Botrytis fuckeliana* (*Botrytis cinerea*). Pages 237-250 *in* T.S. Thind, ed. Fungicide Resistance in Crop Protection: Risk and Management. CAB International, Wallingford, UK.

Annis, S. 2009. Factsheet: Forecasting mummy berry fungus infection. *In* Cooperative Extension: Maine Wild Blueberries. [Online] Available: http://extension.umaine.edu/blueberries/files/2010/06/mummyberry-forecasting-handout.pdf. [January 05, 2015]

Annis, S. and Yarborough, D. 2014. Factsheet: Disease control guide for wild blueberries. *In* Cooperative Extenstion: Maine Wild Blueberries. [Online] Available: http://umaine.edu/blueberries/factsheets/disease/219-disease-control-guide-for-wild-blueberries/. [January 09, 2015].

Annis, S., McGovern, K. and Yarborough, D. 2009. Facsheet: Mummy berry disease forecasting method. *In* Cooperative Extension: Maine Wild Blueberries [Online] Available: http://extension.umaine.edu/blueberries/files/2010/06/mummyberryforecastingmethod.pdf. [January 09, 2015]

Australian Pesticides and Veterinary Medicines Authority (APVMA). 2007. Evaluation of the new active prothioconazole in the product redigo fungicidal seed treatment. ISSN 1443-1335. Australian Pesticides and Veterinary Medicines Authority, Symonston, Australian Capital Territory, Australia.

Batra, L.R. 1983. *Monilinia vaccinii-corymbosi* (*Schlerotiniaceae*): its biology on blueberry and comparison with related species. Mycologia, 75: 131-152.

Batra, L.R. 1988a. Disjunctors in *Monilinia* (Discomycetidae: Sclerotiniaceae) and their significance in classification. Mycologia, 80: 660-664.

Batra, L.R. 1988b. *Monilinia gaylussaciae*, a new species pathogenic to huckleberries (*Gaylussacia*) in North America. Mycologia, 80: 653-659.

Batra, L.R. 1991. World species of *Monilinia* (fungi): their ecology, biosystematics and control. Berlin, J. Cramer , Mycologia Memoir, 16: 246 p.

Batra, L.R. and Batra, S.W.T. 1985. Floral mimicry induced mummy-berry fungus exploits host's pollinators as vectors. Science, 228: 1011-1013.

Brent, K. 2012. Historical perspectives of fungicide resistance. Pages 3-18 *in* T. S. Thind, ed. Fungicide Resistance in Crop Protection: Risk and Management. CAB International, Wallingford, UK.

Brent, K.J. and Hollomon, D.W. 2007a. Fungicide resistance: the assessment of risk. 2nd ed. Fungicide Resistance Action Committee, CropLife International. 53 pp.

Brent, K. J. and Hollomon, D. W. 2007b. Fungicide resistance in crop pathogens: how can it be managed? 2nd ed. Fungicide Resistance Action Committee, CropLife International. 60 pp.

Burgher-MacLellan, K. and MacKenzie, K. 2004. An overview of RAPD analysis to estimate genetic relationshiops in lowbush blueberry. Pages 295-306 *in* C.F. Forney and L.J. Eaton, eds. Proceedings of the ninth American Blueberry Research and Extension Workers Conference. Food Products Press, Binghamton, NY, US.

Burnett, A., Lalancette, N. and McFarland, K. 2010. First report of the peach brown rot fungus *Monilinia fructicola* resistant to demethylation inhibitor fungicides in New Jersey. Plant Disease, 94: 126.

Cox, K.D. and Schem, H. 2001. Gradients of primary and secondary infection by *Monilinia vaccinii-corymbosi* from point sources of ascospores and conidia. Plant Disease, 85: 955-959.

De Waard, M.A., Georgopoulos, S.G., Hollomon, D.W., Ishii, H., Leroux, P., Ragsdale, N.N. and Schwinn, F.J. 1993. Chemical control of plant diseases: problems and prospects. Annual Review of Phytopathology, 31: 403-421.

Delbridge, R. and Hildebrand, P. 1995. Lowbush blueberry factsheet: Monilinia blight of lowbush blueberry. *In* Wild Blueberry Network Information Centre. [Online] Available: http://www.dal.ca/content/dam/dalhousie/images/sites/wild-blueberry/pdfs/Monilinia_Blight_Lowbush_Blueberry.pdf. [January 05, 2015]

Delbridge, R., Hildbrand, P. and Yarborough, D. 1998. Facsheet: A method to control Monilinia blight. *In* Cooperative Extension: Maine Wild Blueberries. [Online] Available: http://umaine.edu/blueberries/factsheets/disease/217-a-method-to-control-monilinia-blight/ [January 19, 2015]

Delbridge, R. and Rogers, D. 2013. Wild blueberry insect and disease management schedule. [Online] Available: http://www.perennia.ca/Pest%20Management%20Guides/Fruits/2013/wild%20blueberry%20I&D%202013.pdf. [January 13, 2015]

Delbridge, R., Rogers, D., Burgess, P., Wood, S. and Leclerc, M. 2015. Guide to weed, insect and disease management in wild blueberry. *In* Fruit Production, Perennia. [Online] Available: http://www.perennia.ca/Pest%20Management%20Guides/Fruits/2015/Wild%20Blueberry%20Pest%20Guide%202015.pdf. [December 05, 2015].

Eaton, L.J., Glen, R.W. and Wyllie, J.D. 2004a. Efficient mowing for pruning wild blueberry fields. Pages 123-132 *in* C.F. Forney and L.J. Eaton, eds. Proceedings of the ninth American Blueberry Research and Extension Workers Conference. Food Products Press, Binghamton, NY, US.

Eaton, L.J., Sanderson, K.R. and Holy, J. 2004b. Effects of salt deposition from salt water spray on lowbush blueberry shoots. Pages 95-104 *in* C.F. Forney and L.J. Eaton, eds. Proceedings of the ninth American Blueberry Research and Extension Workers Conference. Food Products Press, Binghamton, NY, US.

European Food Safety Authority. 2010. Modification of the existing MRLs for propiconazole in table and wine grapes, apples and stone fruits. EFSA Journal. 8: 1824 [32 pp.].

European Food Safety Authority. 2011. Review of the existing maximum residue levels (MRLs) for metconazole according to Article 12 of Regulation (EC) No 396/2005. ESFA Journal 9: 2422 [47 pp.].

European Food Safety Authority. 2012. Reasoned opinion on the modification of existing MRLs for prothioconazole in rape linseed, poppy seed and mustard seed. EFSA Journal. 10: 2952. [35 pp.].

Fletcher, J.T. and Wolf, M.S. 1981. Insensitivity of *Erysiphe graminis* f. sp. *Hordei* to triadimefon, triadimenol and other fungicides. Pages 633-640 *in* Proceedings of Brighton Crop Protection Conference-Pests and Disease. British Crop Protection Council, Croydon, UK.

Fungicide Resistance Action Committee. 2013a. List of plant pathogenic organisms resistant to disease control agents. [Online] Available: http://www.frac.info/docs/default-source/publications/list-of-resistant-plant-pathogens/list-of-resistant-plant-pathogenic-organisms---february-2013.pdf?sfvrsn=4 [February 01, 2016]

Fungicide Resistance Action Committee. 2013b. Pathogen risk list. [Online] Available: http://www.frac.info/docs/default-source/publications/pathogen-risk/pathogen-risk-list.pdf?sfvrsn=8 [February 01, 2016]

Fungicide Resistance Action Committee. 2015. FRAC code list: fungicide sorted by mode of action. [Online] Available: http://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2015-finalC2AD7AA36764.pdf [June 08, 2015]

Gachango, E., Hanson, L.E., Rogas, A., Hao, J.J. and Kirk, W.W. 2012. *Fusarium* spp. causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. Plant Disease, 96: 1767-1774.

Georgopoulos, S.G. 1988. Genetics and population dynamics. Pages 12-13 *in* C.J. Delp, ed. Fungicide Resistance in North America. American Phytopathological Scociety, St. Paul, MN

Golembiewski, R.C., Vargas, J.M., Jr., Jones, A.L. and Detweiler, A.R. 1995. Detection of demethylation inhibitor (DMI) resistance in *Sclerotinia homoeocarpa* populations. Plant Disease, 79: 491-493.

Haidukowski, M. Visconti, A., Perrone, G., Vanadia, S., Pancaldi, D., Covarelli, L., Balestrazzi, R. and Pascale, M. 2012. Effect of prothioconazole-based fungicides on Fusarium head blight, grain yield and deoxynivalenol accumulation in wheat under field conditions. Phytopathologia Mediterranea, 51: 236-246.

Hall, I.V., Aalders, L.E., Nickerson, N.L. and Vander Kloet, S.P. 1979. The biological flora of Canada 1. *Vaccinium angustifolium* Ait., sweet lowbush blueberry. The Canadian Field-Naturalist, 93: 415-430.

Heaney, S.P., Humphreys, G.J., Hutt, P., Montiel, P. and Jegerings, P.M.F.E. 1984. Sensitivity of barley powdery mildew to systemic fungicides in the UK. Pages 459-464 *in* British Crop Protection Conference-Pests and Diseases. British Crop Protection Council, Croydon, UK.

Hildebrand, P.D. and Braun, P.G. 1991. Factors affecting infection of lowbush blueberry by ascospores of *Monilinia vaccinii-corymbosi*. Canadian Journal of Plant Pathology, 13: 232-240.

Holb, I.J. and Schnabel, G. 2007. Differential effect of triazoles on mycelial growth and disease measurements of *Monilinia fructicola* isolates with reduced sensitivity to DMI fungicides. Crop Protection, 26: 753-759.

Hollomon, D.W. 1984. A laboratory assay to determine the sensitivity of *Rhychosporium secalis* to the fungicide triadimenol. Plant Pathology, 33: 65-70.

Hollomon, D.W. 2015. Fungicide resistance: 40 years on and still a major problem. Pages 3-12 *in* H. Ishii and D. W. Hollomon ed. Fungicide Resistance in Plant Pathogen: Principles and a Guide to Practical Management. Springer, Tokyo, Heidelgerg, New York, Dordrecht, London.

Holst-Jensen, A., Kohn, L.M., Jakobsen, K.S. and Schumacher, T. 1997. Molecular phylogeny and evolution of *Monilinia* (Sclerotiniaceae) based on coding and noncoding rDNA sequences. American Journal of Botany, 84: 686-701.

Honey, E.E. 1936. North American species of *Monilinia*. I. occurrence, grouping, and life-histories. American Journal of Botany, 23: 100-106.

Hu, M.-J., Luo, C.-X., Grabke, A. and Schnabel, G. 2011. Selection of a suitable medium to determine sensitivity of *Moniliinia fructicola* mycelium to SDHI fungicides. Journal of Phytopathology, 159: 616-620.

Huggenberger, F., Collins, M.A. and Skylakakis, G. 1984. Decreased sensitivity of *Sphaerotheca fuliginea* to fenarimol and other ergosterol-biosynthesis inhibitors. Crop Protection, 3: 137-149.

Ishii, H. 2012. Resistance to QoI and SDHI fungicides in Japan. Pages 223-234 *in* T.S. Thind, ed. Fungicide Resistance in Crop Protection: Risk and Management. CAB International, Wallingford, UK.

Jensen, K.I.N. and Yarborough, D.E. 2004. An overview of weed management in the wild blueberry-past and present. Pages 229-256 *in* C.F. Forney and L.J. Eaton, eds. Proceedings of the ninth American Blueberry Research and Extension Workers Conference. Food Products Press, Binghamton, NY, US.

Joint FAO/WHO Meeting on Pesticide Residues (JMPR). 2008. Prothiconazole (232). [Online] Available: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation09/Prothioconazole.pdf [November 03, 2015].

Keith, B. 2012. Historical perspectives of fungicide resistance. Pages 3-18 *in* T.S. Thind, ed. Fungicide Resistance in Crop Protection: Risk and Management. CAB International, Wallingford, UK.

Keon, J.P.R., White, G.A. and Hargreaves, J.A. 1991. Isolation, characterization and sequence of a gene conferring resistance to the systemic fungicide carboxin from the maize smut pathogen, *Ustilago maydis*. Current Genetics, 19: 475-481.

Köller, W. 1988. Sterol demethylation inhibitors: Mechanisms of action and resistance. Pages 79-88 *in* C.J. Delp, ed. Fungicide Resistance in North America. American Phytopathological Society, St. Paul, MN.

Köller, W. 1992. Antifungal agents with target sites in sterol function and biosynthesis. Pages 119-206 *in* W. Köller, ed. Target Sites of Fungicide Action. CRC Press, Boca Raton, FL.

Köller, W. and Scheinpflug, H. 1987. Fungal resistance to sterol biosynthesis inhibitors: a new challenge. The American Phytopathological Society, 71: 1066-1074.

Köller, W., Wilcox, W.F., Barnard, J., Jones, A.L. and Braun, P.G. 1997. Detection and quantification of resistance of *Venturia inaequalis* populations to sterol demethylation inhibitors. Phytopathology, 87: 184-190.

Lambert, D.H. 1990. Effects of pruning method on the incidence of mummy berry and other lowbush blueberry diseases. Plant Disease, 74: 199-201.

Lehman, J.S. and Oudemans, P.V. 1997. Phenology of apothecium production in populations of *Monilinia vaccinii-corymbosi* from early- and late-maturing blueberry cultivars. Phytopathology, 87: 218-223.

Lehman, J.S. and Oudemans, P.V. 2000. Variation and heritability of phenology in the fungus *Monilinia vaccinii-corymbosi* on blueberry. Phytopathology, 90: 390-395.

Leroux, P. and Walker, A.-S. 2011. Multiple mechanisms account for resistance to sterol 14α-demethylation inhibitors in field isolates of *Mycosphaerella graminicola*. Pest Management Science, 67: 44-59.

Lockhart, C.L. 1961. *Monilinia* twig and blossom blight of lowbush blueberry and its control. Canadian Journal of Plant Science, 41: 336-341.

Lockhart, C.L., Delbridge, R.W. and McIsaac, D. 1983. Observations on *Monilinia* twig and blossom blight of the lowbush blueberry in the Maritime Provinces. Canadian Plant Disease Survey, 63: 31-34.

Longstroth, M. and Schilder, A. 2013. Managing mummy berry shoot strike infections. *In* Michigan State University Extension. [Online] Available: http://msue.anr.msu.edu/news/managing_mummy_berry_shoot_strike_infections. [July 25, 2016].

Luo, C.-X. and Schnabel, G. 2008. Adaptation of fungicides in *Monilinia fructicola* isolates with different fungicide resistance phenotypes. Phytopathology, 98: 230-238.

Luo, C.-X., Cox, K.D., Amiri, A. and Schnabel, G. 2008. Occurrence and detection of the DMI resistance-associated genetic element 'Mona' in *Monilinia fructicola*. Plant Disease, 92: 1099-1103.

Markey, K.L., Baird, A.H., Humphrey, C. and Negri, A.P. 2007. Insecticides and a fungicide affect multiple coral life stages. Marine Ecology Progress Series, 330: 127-137.

Masner, P., Muster, P. and Schimid, J. 1994. Possible methionine biosynthesis inhibition by pyramidinamine fungicides. Journal of Pesticide Science, 42: 163-166.

McGovern, K.B., Annis, S.L. and Yarborough, D.E. 2012. Efficacy of organically acceptable materials for control of mummy berry disease on lowbush blueberries in Maine. International Journal of Fruit Science, 12: 188-204.

McKeen, C.D., Reilly, C.C. and Pusey, P.L. 1986. Production and partial characterization of antifungal substances antagonistic to *Monilinia fructicola* from *Bacillus subtilis*. Phytopathology, 76: 136-139.

Mclsaac, D. 1997. Wild blueberry fact sheet: Growing wild lowbush blueberries in Nova Scotia. *In* Wild blueberry network information centre. [Online] Available: http://www.dal.ca/content/dam/dalhousie/images/sites/wild-blueberry/pdfs/Growing_Wild_Lowbush_Blueberries_NS.pdf. [May 10, 2016].

Mclsaac, D. and Reid, S. 2000. Wild blueberry fact sheet: Wild blueberry production and marketing in Nova Scotia – A situation report-2000. *In* Wild blueberry network information centre. [Online] Available: http://www.dal.ca/content/dam/dalhousie/images/sites/wild-blueberry/pdfs/A%20Situation%20Report%20-%202000.pdf. [May 10, 2016].

Milholland, R.D. 1974. Factors affecting apothecium development of *Monilinia vaccinii-corymbosi* from mummified highbush blueberry fruit. Phytopathology, 64: 296-300.

Milholland, R.D. 1977. Sclerotium germination and histopathology of *Monilinia vaccinii-corymbosi* on highbush blueberry. Phytopathology, 67: 848-854.

Miura, I., Kamakura, T., Maeno, S., Hayashi, S. and Yamaguchi, I. 1994. Inhibition of enzyme secretion in plant pathogens by mepanipyrim a novel fungicide. Pesticide Biochemistry and Physiology, 48: 222-228.

Munda, A. 2011. *Monilinia* pathogens of cultivated and native *Vaccinium* species in Slovenia. Acta Agriculturae Slovenica, 97: 99-104.

Parker, J.E., Warrilow, A.G.S., Cools, H.J., Martel, C.M., Nes, W.D., Fraaije, B.A., Lucas, J.A., Kelly, D.W. and 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: 1460-1465.

Parker, J.E., Warrilow, A.G.S., Cools, H.J., Fraaije, B.A., Lucas, J.A., Rigdova, K., Griffiths, W.J., Kelly, D.E. and Kelly, S.L. 2013. Prothioconazole and prothioconazole-desthio activities against *Candida albicans* sterol 14-α-demethylase. Applied and Environmental Microbiology, 79: 1639-1645.

Penman, L.N. and Annis, S.L. 2005. Leaf and flower blight caused by *Monilinia vaccinii-corymbosi* on lowbush blueberry: Effects on yield and relationship to bud phenology. Phytopathology, 95: 1174-1182.

Percival, D. and Beaton, E. 2012. Suppression of Monilinia blight: strategies for today and potential fungicide options for tomorrow. International Journal of Fruit Science. 12: 124-134.

Percival, D. and Sanderson, K. 2004. Main and interactive effects of vegetative-year applications of nitrogen, phosphorus, and potassium fertilizers on the wild blueberry. Pages 105-127 *in* C.F. Forney and L.J. Eaton, eds. Proceedings of the ninth American Blueberry Research and Extension Workers Conference. Food Products Press, Binghamton, NY, US.

Ramsdell, D.C., Nelson, J.W. and Myers, R. 1974. An epidemiological study of mummy berry disease of highbush blueberry. Phytopathology, 64: 222-228.

Russell, P.E. 2004. Sensitivity baselines in fungicide resistance research and management. Fungicide Resistance Action Committee Monograph No. 3. [Online] Available: http://www.frac.info/docs/default-source/publications/monographs/monograph-3.pdf?sfvrsn=8 [April 18, 2016].

Sanssené, J., Selim, S., Roisin-Fichter, C., Djerroud, L., Deweer, C. and Halama, P. 2011. Protective and curative efficacy of prothioconazole against isolates of *Mycosphaerella graminicola* differing in their *in vitro* sensitivity to DMI fungicides.

Scherm, H., Ngugi, H.K., Savelle, A.T. and Edwards, J.R. 2004. Biological control of infection of blueberry flowers caused by *Monilinia vaccinii-corymbosi*. Biological control, 29: 199-206.

Schnabel, G., Bryson, P.K., Bridges, W.C. and Brannen, P.M. 2004. Reduced sensitivity in *Monilinia fructicola* to propiconazole in Georgia and implications for disease management. Plant Disease, 88: 1000-10004.

Shinners, T.C. and Olson, A.R. 1996. The gynoecial infection pathway of *Monilinia vaccinii-corymbosi* in lowbush blueberry (*Vaccinium angustifolium*). Canadian Journal of Plant Science, 76: 493-497.

Skylakakis, G. 1987. Changes in the composition of pathogen populations caused by resistance to fungicides. Pages 227-237 *in* M. S. Wolfe and C. E. Caten ed. Populations of Plant Pathogens: Their Dynamics and Genetics. Blackwell Scientific Publications, Oxford, London, Edinburgh, Boston, Palo, Alto, Melbourne.

Stanis, V.F. and Jones, A.L. 1985. Reduced sensitivity to sterol-inhibiting fungicides in field isolates of *Venturia inaequalis*. Phytopathology, 75: 1098-1101.

Thind, T.S., Clerjeau, M. and Oliver, J.M. 1986. First observations on the resistance in *Venturia inaequalis* and *Guignaria bidwellii* to ergosterol-biosynthesis inhibitors in France. Pages 491-498 in Proceedings of British Crop Protection Conference-Pests and Diseases. British Crop Protection Council, Thornton Health, Surrey, UK.

Thompson, A.A. and Annis, S.L. 2014. Fungicide sensitivity assessed in *Monilinia vaccinii-corymbosi* isolates from lowbush blueberry fields in Maine. Plant Health Research, 15: 141-144.

Ulrich, J.T. and Mathre, D.E. 1972. Mode of action of oxathiin systemic fungicides V. effect on electron transport system of *Ustilago maydis* and *Saccharomyces cerevisiae*. Journal of Bacteriology, 110: 628-632.

United States Environmental Protection Agency (USEPA). 2007. Pesticide fact sheet: prothioconazole. (7501 P). Office of Prevention, Pesticides and Toxic Substances, United States Environmental Agency, Washington, DC.

Vander Kloet, S.P. 1978. Systematics, distribution, and nomenclature of the polymorphic *Vaccinium angustifolium*. Rhodora, 80: 358-376.

Villani, S.M. and Cox, K.D. 2011. Characterizing fenbuconazole and propiconazole sensitivity and prevalence of 'Mona' in isolates of *Monilinia fructicola* from New York. Plant Disease, 95: 828-834.

Vinggaard, A.M., Nellemann, C., Dalgaard, M., Jørgensen, E.B. and Andersen, H.R. 2002. Antiandrogenic effects *in vitro* and *in vivo* of the fungicide prochloraz. Toxicological Science, 69: 344-353.

Wharton, P.S. and Schilder, A.C. 2005. Effect of temperature on apothecial longevity and ascospore discharge by apothecia of *Monilinia vaccinii-corymbosi*. Plant Disease, 89: 397-403.

Wise, K.A., Bradley, C.A., Markell, S., Pasche, J., Delgado, J.A., Goswami, R.S. and Gudmestad, N.C. 2011. Sensitivity of *Ascochyta rabiei* populations to prothioconazole and thiabendazole. Crop Protection, 20: 1000-1005.

Wong, F.P. and Midland, S.L. 2007. Sensitivity distributions of California populations of *Coletorichum cereal* to the DMI fungicides propiconazole, myclobutanil, tebuconazole, and triadimefon. Plant Disease, 91: 1547-1555.

Wulff, E.G., Mguni, C.M., Mansfeld-Giese, K., Fels, J., Lubeck, M. and Hockenhull, J. 2002. Biochemical and molecular characterization of *Bacillus amyloliquefaciens*, *B. subtilis*, and *B. pumilus* isolates with distinct antagonistic potential against *Xanthomonas campetris*. Plant Pathology, 51: 574-584.

Yarborough, D. 2015. Fact sheet: Wild blueberry culture in Maine. *In* Cooperative extension: Maine's native wild blueberries. [Online] Available: https://extension.umaine.edu/blueberries/factsheets/production/wild-blueberry-culture-in-maine/ [May 10, 2016].

Zehr, E. I., Luszcz, L. A., Olien, W. C., Newall, W. C. and Toler, J. E. 1999. Reduced sensitivity in *Monilinia fructicola* to propiconazole following prolonged exposure in peach orchards. Plant Disease. 83: 913-916.

APPENDIX A

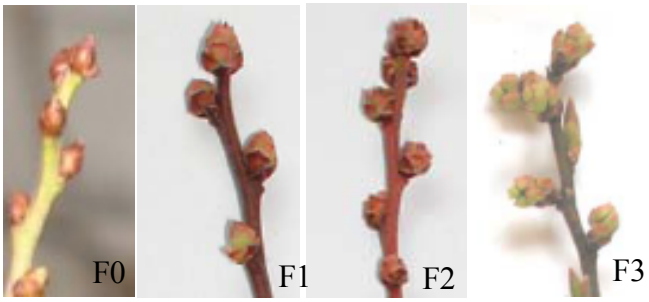


Figure A-1 Lowbush blueberry floral bud stages. F0. Buds tightly closed, no swelling; F1. Buds swelling; F2. Susceptible, bud scales separating; F3. Susceptible, flower bud expand and individual flowers are visible (Annis, 2009)

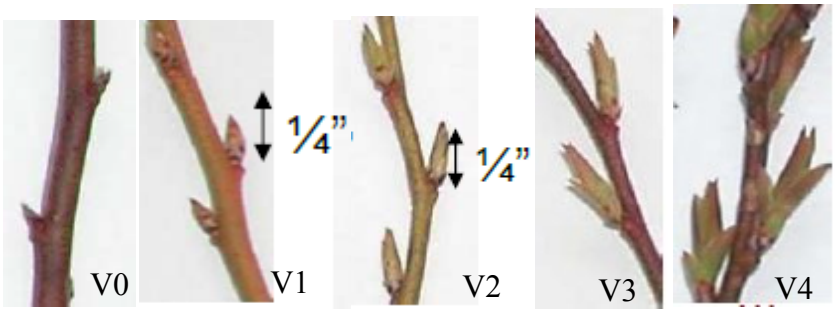
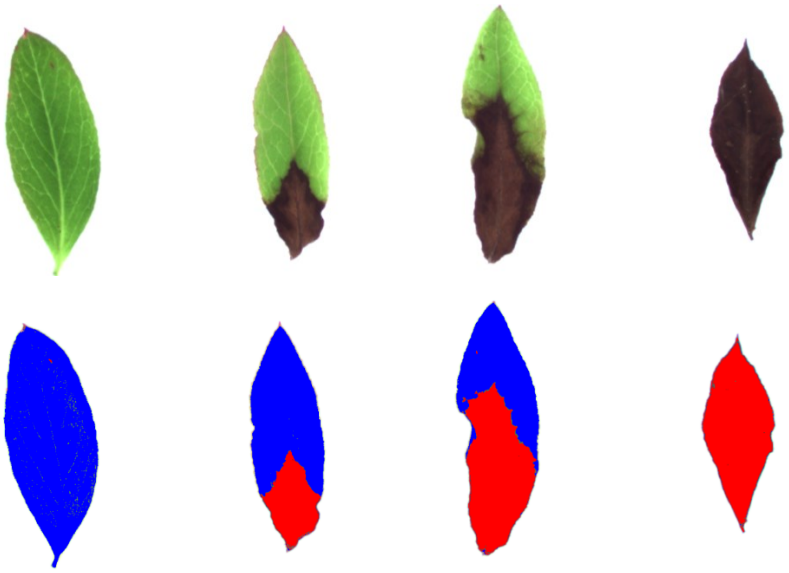


Figure A-2 Lowbush blueberry vegetative bud stages. V0. Buds tightly closed, no swelling; V1. Buds swelling, green tips may be exposed. V2. Susceptible, green buds long than $^1/_4$″; V3. Susceptible, leaf tips are visible and are separating apart; V4. Susceptible, leaves unwrapping and spreading out (Annis, 2009)



Disease progression-

0%	27%	54%	100%

Figure A-3 Disease progression used for disease severity assessment of Monilinia blight infection

Disease progression-

8%	23%	37%	82%

Figure A-4 Disease progression used for disease incidence assessment of Monilinia blight infection

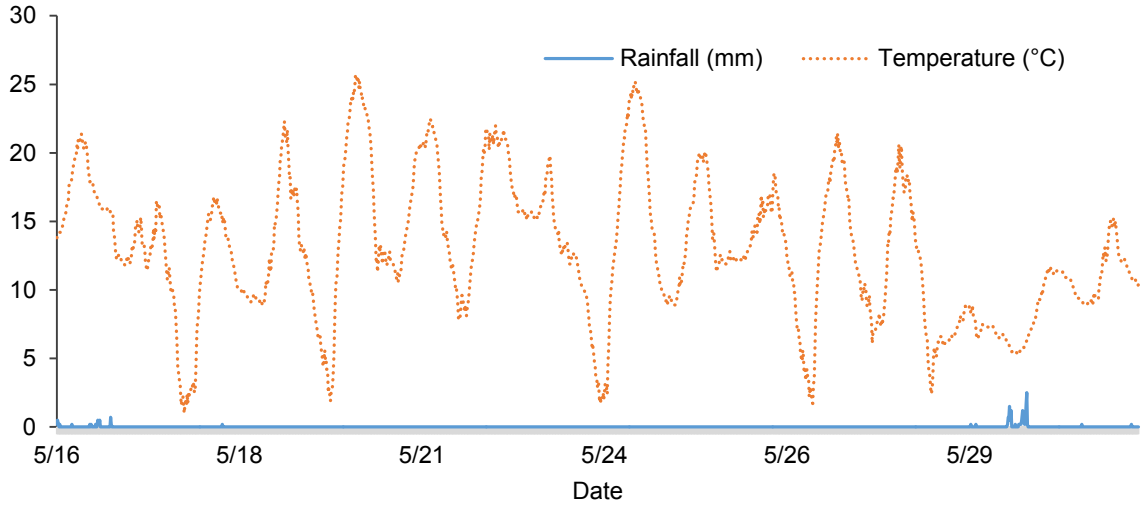


Figure A-5 Environmental conditions in Aulac, NB Monilinia blight control field research trial in 2012

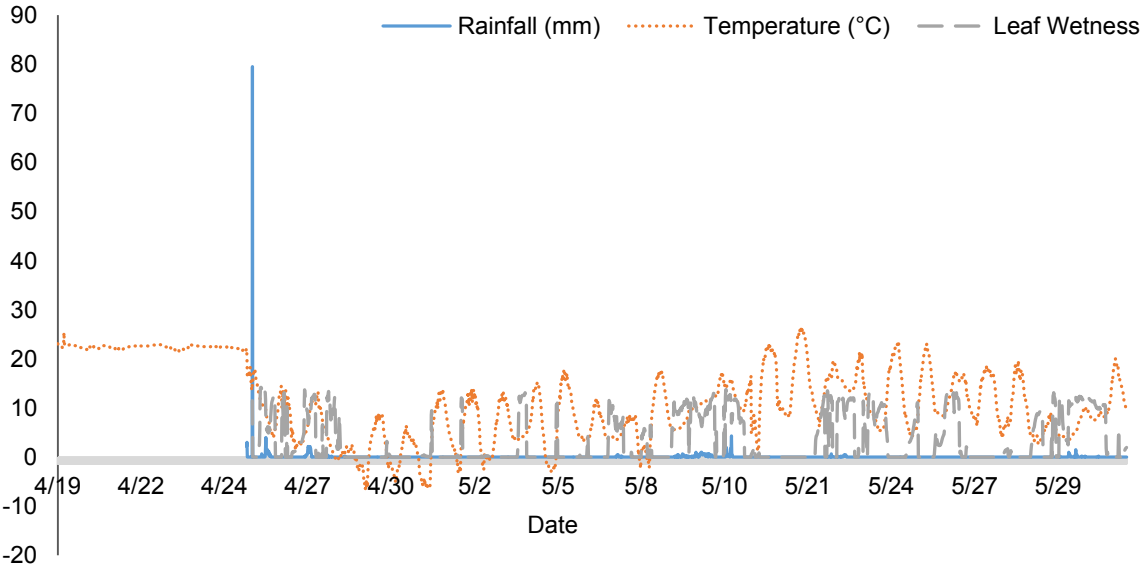


Figure A-6 Environmental conditions in Kemptown, NS Monilinia blight control field research trial in 2012

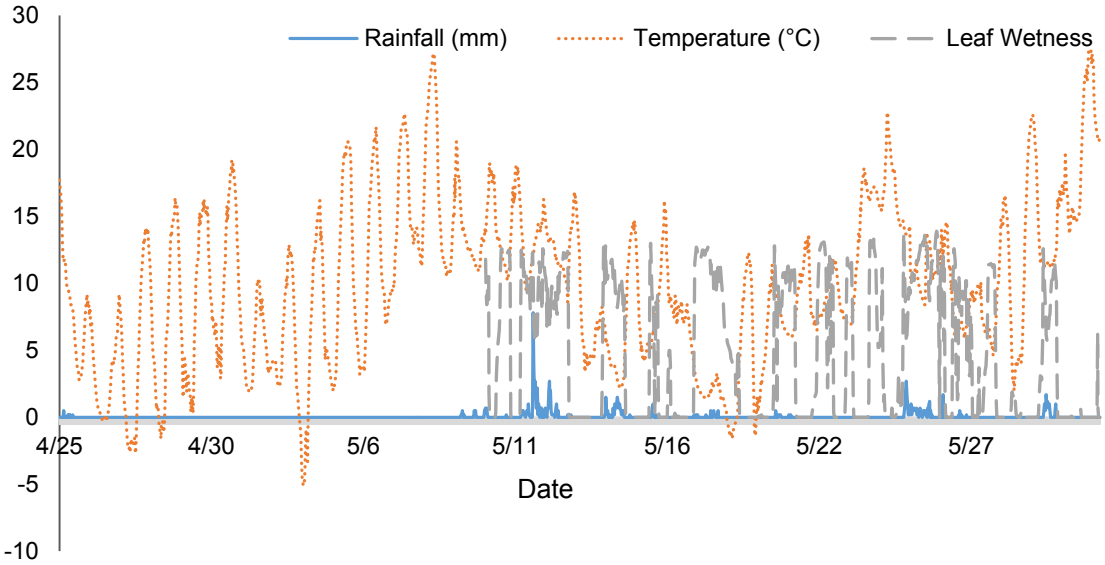


Figure A-7 Environmental conditions in Farmington, NS Monilinia blight control field research trial in 2013

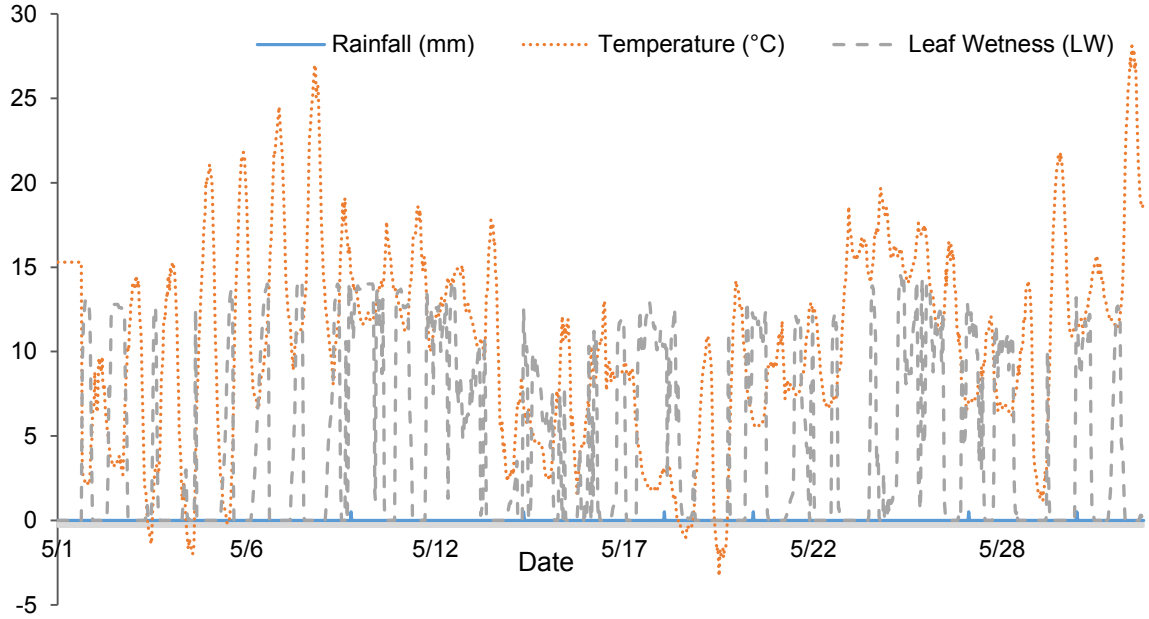


Figure A-8 Environmental conditions in Mt. Thom, NS Monilinia blight control field research trial in 2013