EPIDEMIOLOGY AND MANAGEMENT OF SWEET CHERRY POWDERY MILDEW IN WASHINGTON NURSERIES

By

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A dissertation submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

WASHINGTON STATE UNIVERSITY
Department of Plant Pathology

DECEMBER 2016

To the Faculty of Washington State University

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ACKNOWLEDGMENTS

I would like to convey my deepest gratitude to my advisor Dr. Gary G Grove for his generosity and supporting me all the years of my Ph.D. life. It is hard to convey the gratitude in words about his magnanimity and his inspiring personality. I consider myself lucky to have awesome committee members in my thesis project. I would also like to thank Dr. Scot Hulbert for being my on-campus advisor when I was in Pullman.

I thank Mr. Mark Nelson for his advice and giving suggestions in my research. I appreciate the help of Dr. Claudia Probst, Ms. Robert Boal, Ms. Marcia Walter and Ms. Neusa Guerra. I also thank Mr. Maurisio Garcia and Mr. Jeff Henry (late) for the help in the nursery.

I appreciate the generosity of Dr. Kenneth Eastwell, Dr. Rayapati for allowing me to use some of their lab equipment. I also appreciate Dr. Doug Walsh, Dr. Michelle Moyer and Dr. Markus Keller for letting me use their lab microscopes. I thank Dr. Bernardo Chaves for helping me in constructing the graphs in SAS. I thank the following funding sources for my research; Washington Tree Fruit Research Commission, Oregon Sweet Cherry Commission, Northwest Nursery Improvement Institute and Washington State University Agricultural Research Center.

I appreciate my friends for their emotional support and cheering me up whenever I felt down. I also appreciate my lab crew, Dr. Qingchun Liu and Dr. Hui Yan, for helping me in and making myself familiar to the lab. I convey my gratitude to my friends Dr. John Cramer and his wife Ms. Alice Cramer for helping me in Pullman with their generous gifts and rides to different places. I thank all my Prosser friends of the Plant Pathology department for their time and advice. A simple thanks is not enough for the sacrifices my family made for me during my Ph.D. work. I am very much thankful to my entire family- my parents, in laws, husband, sister and kids.

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Abstract

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Washington State University
December 2016

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Powdery mildew of sweet cherry caused by *Podosphaera prunicola* is a major problem in cherry nursery production. Morphological studies, molecular studies and cross inoculation studies have shown that chokecherry and sweet cherry are infected by two different *Podosphaera* species, and the chokecherry powdery mildew fungus was identified as *Podosphaera tridactyla* based on the descriptions of Braun and Cook (2012). Epidemiological studies that were conducted in the sweet cherry nursery located in Quincy WA, have indicated that the initial inoculum might be originating from the nearby cherry orchards. Cherry rootstock had shown chasmothecia on the stem (with 2-5% viable ascospores) but the rootstock did not develop powdery mildew signs. Powdery mildew infections in the nursery start after 1 month of powdery mildew epidemic in the nearby orchards. Spore concentration in the nurseries was monitored using Rotary impaction spore traps and *P.prunicola* spores were detected in the nursery air one month prior to the development of powdery mildew signs in the nursery. Disease ratings have

shown that the incidence of powdery mildew increases from late July until August. There was a positive correlation between the spore concentration, relative humidity and disease severity.

The trees in the storage were loaded with chasmothecia along the stem; the viability of the chasmothecia was high in the month of January and went down by the end of May. These cherry trees neither developed any flag shoot nor developed the signs of powdery mildew. Cherry leaves that were collected from the nursery floor showed the highest viability in the beginning of the year but the viability went down by June, and the chasmothecia with viable ascospores did not develop infections on either the detached leaf or the attached leaf assay. Electrolyzed oxidized water was found to be effective in reducing the disease severity and number of chasmothecia formation ($p<0.05$), whereas the bio fungicides were not effective in controlling powdery mildew in sweet cherry.

TABLE OF CONTENTS

Page

LIST OF TABLES

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

LIST OF FIGURES

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

PREFACE

The chapters included in this dissertation have been prepared for submission to Plant Disease and Plant Health Progress. Citations within each chapter refer to the "Literature cited" section of the chapter and follow the format used in Plant Disease

Dedication

This dissertation is dedicated to my advisor Dr. Gary Glenn Grove

CHAPTER ONE

GENERAL INTRODUCTION

Sweet cherry

Sweet cherry (*Prunus Avium* L,.) belongs to the family *Rosaceae* and is widely cultivated in the temperate regions. It is believed that the cherries might have originated in the region between the Black Sea and the Caspian Sea in Asia Minor (Fernandez I Marti et al., 2012). Sweet cherries are commercially cultivated in more than 40 countries worldwide, and are being cultivated in temperate, Mediterranean and even subtropical regions. Cherries are a temperate crop that requires both a warm growing season and a winter dormant period. Proper sunlight and warm temperatures are required for the growth of the plant (Webster, A. D and Looney, N.E 2006). The largest producers of sweet cherries are France, Germany, Italy, Spain, Switzerland, Turkey and the USA (Webster, A.D and Looney, N.E., 2006).

Cherries are mainly grown for food. Other uses of the cherry tree include as ornamental trees for timber and woodturning, wardrobe making and musical instruments, and for medicinal purposes. With recent growth in health consciousness, cherry has become more popular as research has shown that the cherry fruits are loaded with phytonutrients, which are beneficial to health (Villamor, D.V., 2012). Cherries have a low Glycemic Index (GI) and are rich in phenolic compounds such as anthocyanins, quercetin and ellagic acid, which have antimicrobial and antioxidant properties and in the prevention of heart disease and certain cancers. Cherries are rich in potassium, which lowers the risk of hypertension, stroke and controls blood pressure. Cherries are a good

source of melatonin which promotes good sleep and fights jetlag, and has anti-inflammatory properties that may help to control gout and arthritis (Duyff, R.L., 2009).

Early European settlers in the 1600's brought cherries to the USA (the history of cherries from Internet), the Pacific Northwest turned out to be nation's leading sweet cherry production areas (Long, L. E., 2001). The climate of the US Pacific Northwest is suitable for cherry production; Washington State is the leading US producer of sweet cherry with 50.9 % of sweet cherry production in the entire US. According to the USDA-NASS tree fruit census report, there are 9 million cherry trees in the ground and during the period of 2002 to 2012, there was an increase of 14,000 acres in the US to the existing cherry acreage. The same report also mentioned that up until 2011, around 38,115 acres were involved in sweet cherry production. In Washington State, sweet cherry production regions are located east of the Cascade Mountains, mainly in the Yakima valley, the Columbia basin and Wenatchee. Yakima is the leading County in sweet cherry production, other Counties involved in sweet cherry production include, Chelan, Benton, Grant, Douglas, Franklin and Okanogan (Smith et al 2003). In the year 2015, WA State produced 237,000 tons of sweet cherries (USDA press release, NASS reports). In WA State, the annual farm gate value of sweet cherry is predicted to be between $130 and $ 180 million. $1/4^{th}$ of the sweet cherry crop is exported from WA State (Smith et al 2003).

History of sweet cherry nurseries in Washington State

The history of cherry nursery industry in the Washington state dates back to 1938, with C&O nursery started producing sweet cherry plants. Most of the nurseries are located in the Quincy and Moses Lake, WA area. Fertile, sandy loam soils with irrigation facilities and mild winters make these areas suitable for the cherry nursery production.

By 2000, the cherry nursery industry had exploded as prices of apples went down. The cherry trees from the US are marketed not only to other states of the US but also to Canada and Mexico (personal communication with Mr. Peter Van Well, owner of Van Well nursery).

Washington nurseries sell about 500,000 cherry trees each year. The farm gate value would be over $5 million annually. The price of a single cherry depends on the rootstock. In 2010 more cherry trees were being sold each year and there is scope for more cherry trees to be produced in the future (Personal communication with Mr. Bill Howell, Northwest Nursery Improvement Institute).

Commercially grown cherry trees are either budded or grafted. The part of the tree that lies above the graft / bud union is referred as the scion and the part that lies below the graft / bud union is known as the rootstock (Long, L.E, Kaiser, C., 2010). The scion variety that is grafted on to Mazzard rootstock is good for deep loam soils. It is believed that more than 2400 years ago, Mazzard seedlings were used as rootstock for cherry production (Webster, A.D and Looney, N.E., 2006). Mazzards are easy to raise from seed, and not only provide good graft compatibility to both sweet and sour cherry cultivars but also bestow long life to the trees. Burrowing animals such as gophers show less preference towards Mazzard roots. (Webster, A.D and Looney, N.E., 2006). Mazzard performs well in a wide variety of soils from sandy-loam to clay-loam. Like any other rootstock its performance is poor in wet soils and poorly drained soils. It shows vigorous growth and offers premium quality fruit with moderate inputs of pruning and management (Long, L.E., Kaiser, C., 2010). The limitations of Mazzard include lack of precocity and poor perfomence in wet soils (Long, L.E 2009).

Mahaleb is also used as rootstock, a more recent practice compared with Mazzard. In the mid 1800's Mahaleb rootstock became prominent in the USA, as it is easy to germinate from seed and resistant to leaf spot disease, and also renders vigorous growth. However, research conducted in the mid 1920's showed that trees on Mahaleb rootstock had a short life span, and nurserymen shifted back to Mazzard rootstock. Mahaleb renders trees of a short stature compared to Mazzard, and can grow well in wet soils. Mahaleb is commonly used as a rootstock for sour cherry culture (Webster, A.D and Looney, N.E., 2006). The vigorous rootstock is useful for the trunk-shaking method of harvesting. Rootstocks chosen from Mahaleb served superior agronomic qualities for *Verticillium* wilt; fewer attempts were made in achieving wilt resistant rootstocks (Snyder et al 1981).

Gisela is a dwarf rootstock and it provides high precocity and productivity compared to Mazzard rootstock (Robert, L. A., Lang, A.G., 1999). The tree size is up to 40 % smaller compared to Mazzard depending on the type of Gisela rootstock used. 'Gisela 5', 'Gisela 6' and 'Gisela 12' are commercially available rootstocks. In the Pacific Northwest it was observed that 'Gisela 5' reduces plant vigor by up to 60 % when compared to 'Mazzard' seedlings. Gisela 6 is well adapted for wide range of soil types but the tree requires anchorage on windy sites in the PNW. Gisela 12 also adapted to a wide range of soils and has proven to be precocious and productive (Long, L.E 2010).

Colt rootstock variety operates will in replant conditions and non-fumigated soils. Krymsk 5 is another rootstock variety originated from Russia. It perfroms well hot as well as cold climates (Long, L.E 2009).

Figure: 1a Mahaleb seedlings

Figure: 1b Mazzard seedlings

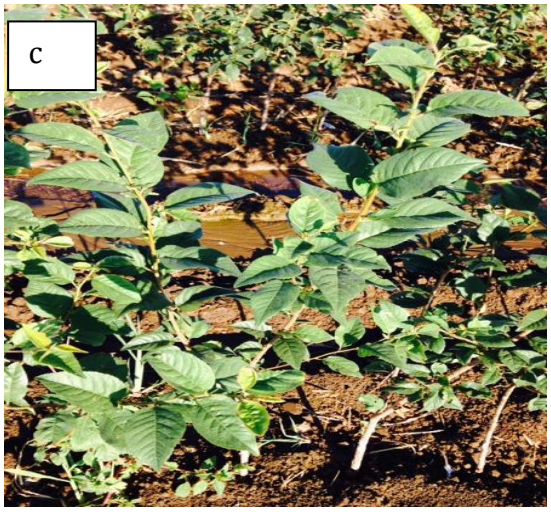

Figure: 1c Gisela 12 seedlings

Figure 1d: Gisela 6 seedlings

Bing (grafted as scion on the rootstock) is the most prominent cultivar of sweet cherry grown in the USA specifically in the Pacific Northwest states. It was first introduced to the PNW by Seth Lewelling and named after the foreman of Chinese work

crew (McClintock, T.C., 1967). It originated from a seedling of the cultivar Black Republican in 1875 in the Lewelling nursery of Milwaukee, Oregon, USA. Bing trees grow vigorously and blossom early to midseason. Bing variety provide moderate yields, more productive than the variety Regina and less productive than Sweetheart (Long et al 2014). Bing is the most popular variety and used as a standard varity for analysis of other sweet cherry varieties (Wendy, J.E., 2015). The fruits are large and red-black in color with very firm, crispy dark red flesh. It is a fruit famous for export and being shipped to different countries of the world (Webster, A.D and Looney, N.E., 2006).

Dark red sweet cherry fruits are dominant varieties in the US market; cultivar Rainier is a yellow color variety and was produced at the cherry breeding program of Washington State University, Irrigated Agriculture Research and Extension Center, Prosser in 1952 by Harold fogle and was released to the market in 1960. The red blush to the fruit is due to the sunlight (Chou, H.C., 2001). Other common varieties that are commonly grown in Washington State are Van, Lapines, Chelan, Lambert and Sweet heart (Smith et al 2003).

For rootstock production, seeds are planted in the fall of the first year of a cherry nursery's production. The Mazzard tree used for the production of seeds and the tree used for bud production are checked for viral infection each year by the National Clean Plant Network (NCPN), thus the trees are produced free of virus (Pete Vanwell personal communication). The seed remains in the soil and gets stratified during winter and in the following year's spring; the seed germinates the seedlings will be dug up in the fall of the 2nd year; any broken plants without roots will be removed and the healthy seedlings kept in storage. The seedlings will be replanted in a different field in the spring (April) of the

subsequent year, which will be 3rd year for the seedlings. These plants are called liners as they are lined in a row 6" apart. In the fall of the same year (August) the cherry seedlings are budded with a scion, which could be either Bing or any other desirable cultivar. In the spring of following year (the 4th year), the bud grows and the shoots of the rootstock are removed to allow growth of scion. During this growing season the trees reach their maxium size in the nursery (Personal communication with Mr. Bill Howell, Northwest Nursery Improvement Institute).

In an alternative method, shoots of sweet cherry such as Gisela are planted by dipping them in rooting mixture, instead of planting seed. These will be acting like rootstock and once it establishes in the ground by developing roots; bud (scian) is grafted on to the rootstock. The bud grows in the spring of next year and the shoots of the rootstock are removed and the plants are harvested in late- fall of next year.

At the end of fall (usually harvesting is carried out in the month of November and December) several thousand 4th-year cherry trees are harvested using digger machines, after the seedlings go dormant but before the roots freeze in the ground, seedlings will be sent to the barn. The bare roots of the seedling will be moistened and graded by stem caliper, and the seedlings are bundled in groups of 5–10. The seedling bundles are stored in storehouses and kept in storage during winter. In early spring of the following year the seedlings' roots will be covered with moist wood chips and wrapped with plastic sheet to be distributed to the growers (personal communication with Pete Vanwell).

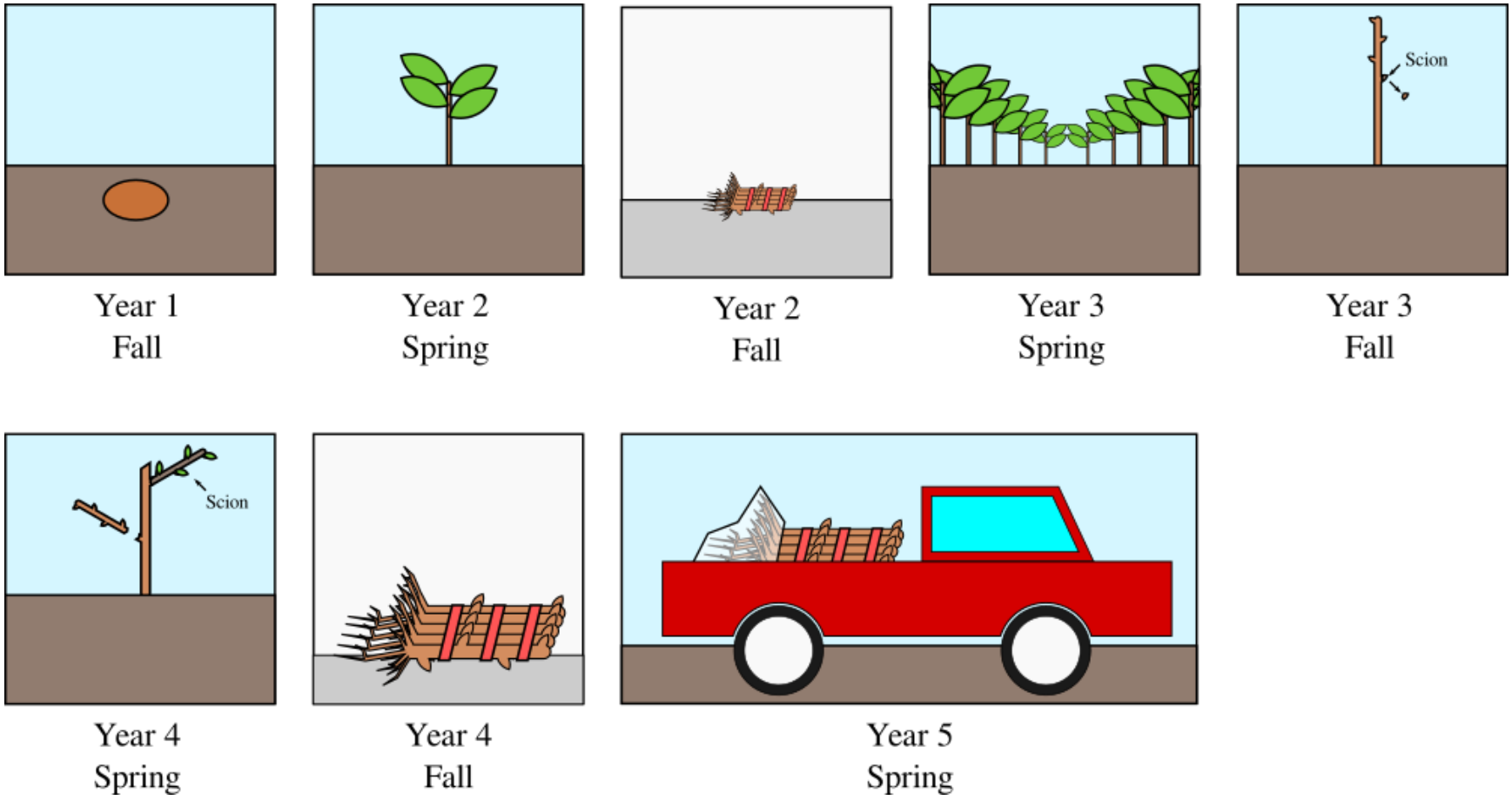

Figure 2: Diagrammatic representation of sweet cherry nursery production; figure generated using Inkscape version 0.92 (www.Inkscape.org)

Powdery mildews are the most easily recognizable pathogenic fungi; the common name powdery mildew is a reference to the prolifically produced conidia that gives the mildew its powdery appearance. The fungus is capable of infecting nearly 10,000 species of angiosperms (Braun, U., 2002). It is an obligate pathogen and infects leaves, stems, flowers and fruits (Belanger et al 2002). Powdery mildews have been an experimental pathogen used to study various aspects of plant disease etiology, epidemiology and control. Attempts to grow powdery mildews on artificial medium have not been successful, but the pathogens can be grown on the detached leaves of their specific hosts (Glawe, D.A., 2008).

Not all the geographic regions of the world were explored enough for the distribution of the powdery mildews; this includes North and South America, Africa and Asia. Erysiphales are most diversified in the temperate regions of the Northern Hemisphere (Amano K1986). Recent climatic changes in Europe have resulted in numerous first reports of powdery mildews on the continent (Bolay et al 2005). New crop introductions to a region can also result in first reports of powdery mildews (Ale-Agha N, Braun U, Feige B, Jage H. 2000, Kiss L, Vajna Fischl G. 2003, Milevoj L. 2004, Zimmermannova -Pastirc a kovaK, Adamska I, Blaszkowski J, Bolay A, Braun, U. 2002). Studies have also shown that air pollution affects powdery mildew diversity and severity; powdery mildew severity was higher in the suburban regions of Poland compared to the urban regions (Dynowska, M., 1994).

Powdery mildew of sweet cherry

Powdery mildew of sweet cherry in the USA is caused by *Podosphaera prunicola,* previously known as *Podosphaera clandestina* synonym *Alphitomorpha*

clandestina; *P.oxyacanthae*. The genus *Podosphaera* mainly infects woody hosts. *Podosphaera clandestina* is now limited to the fungus that infects *Crataegus* (Braun,U; Cook, R.T.A., 2012). It is an Ascomycete in the order Erysiphales and belongs to the tribe Cystotheceae. The tribe Cystotheceae includes two sections: the tree-parasitic section *Podosphaera* and the herb-parasitic section *Sphaerotheca*. Section *Podosphaera* has about 250 host plants and 86% of them belong to the family *Rosaceae* (Matsuda, S., Takamatsu, S., 2000).

Figure 3: Sweet cherry cultivar Bing, heavily infested with powdery mildew

Signs of sweet cherry powdery mildew are in the form of white mycelium that grows on both sides of the leaf and produces numerous spores. In North America, Galloway first reported cherry powdery mildew in 1888. In 1947 powdery mildew infection on sweet cherry fruit was first observed in Washington State (English, W.H., 1947).

Epidemiology of sweet cherry powdery mildew

Powdery mildews have various strategies for perennation between different growing seasons. *Podosphaera clandestina* (now prunicola) overwinters as chasmothecia in the senescent cherry leaves, bark fissures and on tree crochets (Grove, G.G., and Boal, R.J., 1991b). The chasmothecia formed in the previous season will act as a source of inoculum upon bud break in the subsequent season (Grove, G.G., and Boal, R.J., 1991b). Studies conducted on the dormant buds of nursery stock did not show any flag shoot emergence, indicating that the mycelium does not perennate in the dormant buds. The viability of chasmothecia from the senescent leaves ranged from 55–90%. Chasmothecia of *P. clandestina* (now prunicola) were detected in the orchard air samples indicating that these overwintering structures could be wind dispersed (Grove, G.G and Boal, R.J., 1991b). The same authors reported that they have observed viable overwintered chasmothecia on the young leaves immediately after an air-blast fungicide application.

In the event of rain or irrigation the chasmothecia of *P. prunicola* release ascospores. The effects of temperature and relative humidity on the ascospore release have been evaluated in the laboratory conditions. Temperatures ranging from 5°C – 30°C and at least one hour of free standing water are required for ascospore release (Grove 1991); high moisture alone was not enough for ascospore release (Grove 1991). The optimal temperatures for ascospore release are between 15°C and 20°C. Rainfall greater than 2.8 mm and a continuous wetness for 1 hr after a rain event induced ascospore release in the orchard, as detected by sampling the orchard air (Grove, G.G., 1991). This hints that free water is required for *P. prunicola* ascospore release. Distinct colonies of powdery mildews were first noted between late April and early May in the

Pacific Northwest shortly after bud burst and there is no record of blossom infections in sweet cherry.

The ascospore forms a germ tube and an appressorium is formed at the end of the germ tube. A haustorium will arise from the appressorium and will penetrate into the host's epidermal cell wall; it is the only part that enters the host tissue. Mycelium is amphigenuous, thin and forms irregular patches. In cherry, the powdery mildew lesions are first observed on the abaxial surface of the leaf. In *P. prunicola,* the base of the conidiophore is straight and chains of conidia are formed in a basipetal manner; conidia are of ellipsoid to ovoid shape. Conidia are hyaline uni-nucleate, contain fibrosin bodies and possess large amounts of water. Conidia are dispersed either singly or in short chains, and grow radially like other fungi (personal observation). Conidia cause secondary infections when they reach host tissue and thus lead to a polycyclic epidemic. It has been demonstrated that low vapor pressure deficit and temperatures ranging from 15°C – 25°C promoted the latent period of *P. prunicola* (Grove, G.G, Boal, R. J., 1991). Studies conducted on *P. prunicola* mature colonies showed that the optimum temperature and relative humidity for sporulation were 22°C and 85% respectively. Up to 8 hrs of dark period with a temperature of 10°C and relative humidity of 70–90% favor conidial production. It was shown that 24 hours of incubation with continuous 80% humidity suppressed conidia sporulation. Thus under lab conditions it was shown that relative humidity plays crucial role in the development of powdery mildew (Yan H, 2015). Conidia can germinate immediately when the favorable conditions are met and start the mycelium on the host surface, with conidiophore initiation starting on the 4^{th} day of conidial germination and conidiogenesis beginning on the 5^{th} day. By the seventh day

post-infection, the symptoms can be seen with the unaided eye. Thus the latent period in sweet cherry powdery mildew is 7 days (personal observation).

The concentration of airborne conidia of *P. prunicola* is positively correlated with wind speed and temperatures, whereas relative humidity has the opposite effect on the conidial concentration (Grove 1998). Studies have shown that powdery mildew shows circadian rhythms in conidia formation (Yarwood 1936b). Air sampling data have shown that *P. prunicola* also showed a diurnal pattern, wherein the highest number of conidia were found in late morning and in early afternoon (Grove 1998). Presence of excessive water has been shown to be harmful to the development of powdery mildew; colony development was hindered by rain and by water sprays (Longree, 1939, Yarwood 1939b). Vigorously sporulating colonies of *Blumeria graminis* form clumps of spores and are less prone to dissemination (Hammett, K.R.W., Manners, J.G., 1973). It was reported that the conidia of *B. graminis* traveled from the British Isles to infect plants in Denmark nearly 700 km away (Hermansen et al 1978). Conidia of *Golovinomyces cichoracearum* (DC) were reported to be dispersed 200km in California (Schnathorst, W.C., 1959)

Cherry nurseries are infected by various fungal, bacterial and viral pathogens; powdery mildew is one of the major fungal problems faced by the cherry nurseries. At the younger seedling stage, this epidemic is favored in the nursery production as it reduces the growth and vigor of the seedlings (Personal communication with Pete VanWell). In the later seedling stages powdery mildew is major problem as this foliar infection reduces photosynthesis and stimulate early senescence.

Detection of powdery mildew inoculum using air samplers

Plant pathogens disperse in air and can reach new susceptible host either with in the same filed or in different field or entirely a different continent (Pady & Kapica, 1955; Gregory 1973; Brown And Hovmoller, 2002). Air dispersal is one of the major mechanisms of spread by the plant pathogens. In short distance dispersal, pathogens cause disease epidemics by infecting the new susceptible plants in the same field or adjacent fields. In the long distance dispersal, either the spores of pathogens or pathogens them self can travel to new countries or even continents enduring the cold temperatures, UV light and desiccation and can cause disease in the new locations (Morris et al, 2013; Gregory, 1952). Air sampling devices such as Burkard 7 day spore trap collects the airborne pollen, fungal spores and had provided valuable information in the field of plant pathology (West, J.S., Kimber, R.B.E., 2014). Burkard volumetric spore trap impinges spores on a sticky matrix; the spore identification and enumeration are carried out by visual inspection. The disadvantages of using the Burkard volumetric spore traps include, 1. Powdery mildew conidia are not easy to recognize from one species to another, 2. High number of different species conidia can be harbored with in the same field 3. Enumeration of conidia is laborious and inaccurate often demands several hours of time for the evaluation of individual samples 4. The conidia of the powdery mildew are not clearly identified due to dust, pollen and other debris that is being deposited on the sticky matrix (Peetz, A.B., 2007). These problems can be avoided by using polymerase chain reaction (PCR) based approaches, which detect and quantify DNA from the pathogen by using species-specific primers.

Numerous pathogens were detected and quantified using PCR methods from the environmental samples (Bates, J., et al 2001). ITS region of the rDNA is conserved and it is variable enough to aid in the phylogenetic studies of the closely related species (Hirata, T., Takamatsu, S.,1996). This region has advantages owing to its repetitive nature, stability and heterogeneous among species and have shown near homogeneity within species (Lee and Taylor 1990). Using the species-specific primers for *Erysiphe necator*, amplification was reported from conidia as low as 10 (Falacy et al. 2007). Detection and quantification of *Podosphaera macularis* (hop powdery mildew) was achieved using the internal transcribed spacer region (ITS region) of ribosomal DNA in the laboratory setting with 1000 spores (Peetz, A.B., 2007). Limited success was achieved using the cherry powdery mildew species-specific primers (for the ITS region) in the quantification of the pathogens on the surface of the fruits (Calabro, J. M., 2007).

ITS region is not suitable if there is limited variation with in a genus and also not advisable to use this region while using SYBR green based assay (Chilvers, et.al 2007). Intergenic spacer region (IGS) of rDNA showed more heterogeneity than the ITS region in some pathogenic fungi (Pantou et al 2003, Pramateftaki et al 2000). A successful quantitative real time PCR seed assay for *Botrytis* spp. was developed using IGS primers (Chilvers et al 2007).

Management of cherry powdery mildew

Resistance is the best method of controlling the powdery mildew, planting the powdery mildew resistant varieties is one of the better management tactics. Among the major cultivars of sweet cherry varieties ‘Bing’ is highly susceptible, ‘Van’ and ‘Rainier’ are moderately susceptible to *P. prunicola*. Very few resistant varieties are available that

meet growers need of consistent yield and desirable fruit quality for fresh market. (Olmstead et al 2000a). Tom Toyama at WSU-IAREC identified a sweet cherry variety PMR-1, immune to powdery mildew in 1977. (Olmstead, J.W., Lang, A.G., Grove, G.G., 2001). PMR-1 resulted from open pollinated cherry varieties; the fruit quality of this variety is inferior to the currently growing cultivars. Resistance against powdery mildew in PMR-1 resulted due to a single gene (Olmstead, J.W., Lang, A.G., Grove, G.G., 2001). Fruits of Venus, Chelan, Moreau and Hedelfingen varieties showed resistance towards *P.prunicola,* even though the foliage is susceptible to the infections. (Personal communication with Dr. Oraguzie, cherry breeder at WSU- IAREC).

Control of powdery mildew using chemicals

Fungicides are commonly used for controlling the plant pathogenic fungi. Currently many fungicides are available with different modes of action. Fungicides are categorized based on the mode of action, some with single site mode of action and some with multiple sites mode of action. Fungi are more prone to develop resistance with single site mode of action. Powdery mildew fungi can overcome the fungicide sensitivity due to the polycyclic nature of the epidemic and other aspect that makes powdery mildew resistant to fungicide is genetic recombination. Sterol demethylation inhibitors (DMI's) are the most common fungicides that have been registered for use on sweet cherry powdery mildew. Strobilurins (also known as QoI inhibitors) are no longer used to control the powdery mildew of cereals and cucurbit crops (Belanger, R.R, Bushnell, W.R., Dik, A.J., Carver, T.L.W., 2002). Fungicide resistance against a DMI- triadimefon was identified in the grape powdery mildew isolates in California; the resistance

increased over 12 years in *E.necator* population isolates with little or no use of DMI triadimefon (Miller, T.C., Gubler, W.D., 2004).

Sulfur compounds were used to control the cherry powdery mildew before the 1990's, even though sulfur compounds have the multi site mode of action and a short residual activity, the limitations such as restricted temperature range, significant phytotoxicity risks and interference with other integrated pest management programs have limited its use in Eastern Washington. In late 1980's DMI fungicides were used to control the powdery mildew in the cherry orchard and nursery, ultimately became the exclusive fungicides to control the powdery mildew in the state of Washington (Grove, G.G., Boal, R.J., Bennett, L.H., 2000). By mid 1990's reports of DMI related control failures were encountered (Grove, G.G., 2000).

Management of powdery mildew control with out chemicals

Powdery mildew control with fungicides leads to a potential risk of developing resistance to the chemicals (McGrath, M.T. 2004). Considerations toward environment favor alternatives for the chemical pesticides and there are products or agents that had shown their effectiveness against the powdery mildews (Belanger et al 2002). There are some products that are natural as well as synthetic that can induce resistance in the plants. Natural products such as milk, neem kernels, plant extracts from plants such as *Reynoutrica sachalinensis* and compost extracts were proved to be effective against powdery mildew. These products can induce resistance in the plant against the fungus or can act as protectant. Some living organisms such as bacteria, fungi and arthropods were reported as potential biological agents against the powdery mildew (Belanger et al 2002). A Gram-positive bacteria *Bacillus subtilis* and its different strains have been shown to

produce various antibacterial and antifungal antibiotics such as Zwittermicin, kanosmine and lipopeptides of the surfactin, iturin and fengycin families all of these have antifungal activities (Raaijmakers et al 2008, He et al 1994, Kim, P.I., 2010)

Electrolyzed water (EO) has been in use in Japan food industry for killing the bacteria and parasites on raw fish. Electrolyzed water had been proven to be bactericidal on many pathogenic bacteria such as *Escherichia coli*, *Listeria monocystogenes*, *Bacillus cereus* and *Salmonella* spp. (Kim et al 2001, Venkitanarayanan et al 1999). In vitro experiments have shown that EO water was effective in killing *Botrytis, Monilinia* species in less than 30 sec, where as thick walled pigmented fungi, such as *Curvularia, Helminthosporium* took 2 min or longer to reduce the rate of germination (Buck, J.W., 2002). EO water had significantly reduced the powdery mildew severity when sprayed 2 times a week then continued spraying every other week alternating with other synthetic fungicides (Mueller et al 2003). The exact mechanism of EO water for microbicidal inactivation is not completely known, but high oxidation-reduction potential has been proposed (Kim et al 2000).

Specific objectives of this research are to:

a. To determine whether the powdery mildew pathogen that infects *Prunus virginiana* is same as the one that infects *Prunus avium*, and to check the evolutionary relationship of these two pathogens.
b. To determine the source of primary inoculum in the nurseries
c. To investigate the effects of meteorological factors on powdery mildew epidemics in cherry nurseries

d. To investigate the perennation of *P. prunicola* in the dormant buds of cherry rootstock seedlings

e. To determine the significance of chasmothecia on infested leaf detritus in the nursery

f. To determine the effectiveness of bio fungicides, mineral oil/ paraffinic oil, and EO water in managing the powdery mildew and suppressing chasmothecia numbers.

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CHAPTER TWO

CHOKECHERRY AND SWEET CHERRY ARE INFECTED BY TWO HOST-SPECIFIC *PODOSPHAERA* SPECIES

Abstract

Powdery mildew of sweet cherry is a major problem in the cherry growing regions of Washington State. It is not known whether the powdery mildew that infects sweet cherry is same as the one that infects chokecherry. Morphological features of both powdery mildew fungi were compared, the chasmothecial appendages of the sweet cherry powdery mildew fungi were arranged equatorially, whereas the chasmothecial appendages of the chokecherry powdery mildew fungi were fasciculate. Based on this major difference, it was determined that the powdery mildew that infects chokecherry is *Podosphaera tridactyla* and the powdery mildew that infects sweet cherry is *Podosphaera prunicola* as these features closely match with the description of Braun and Cook 2012). Isolates of *P. tridactyla* and *P. prunicola* formed separated clades, in a phylogenetic tree generated with ITS + LSU sequence of rDNA region. Neighbor-joining analysis of ITS sequences of *P. tridactyla* from chokecherry showed close evolutionary relationship with the fungi infecting sweet cherries rather than the powdery mildew fungi that infect other wild cherry varieties. Cross inoculation experiments also showed that, isolates of powdery mildew from chokecherry did not infect sweet cherry and vise-versa indicating that these fungi are specialized to their hosts. Morphological, phylogenetic and pathological evidence support the presence of two different species of *Podosphaera* that infect chokecherry and sweet cherry in Washington State.

Introduction

Podosphaera prunicola and *Podosphaera tridactyla* (Braun and Cook 2012 It is same all hereinafter) are the causal agents of powdery mildew epidemic in sweet cherries (*Prunus avium*). Studies conducted by Takamatsu et al (2010) have shown that the *Podosphaera clandestina* ITS sequences from *Amelanchier, Crataegus, Prunus* and *Spiraea* sequences did not form a single clade. The sequence isolates of powdery mildew from the *Crataegus* formed a distinct clade and the sequence isolates from *Amelanchier, Prunus* and *Spiraea* were closely related to one another. Thus *P. clandestina* on *Crataegus* was determined as a distinct species from the powdery mildew isolates that were collected from the hosts *Prunus* and *Spiraea* in America and Asia (Braun and Cook 2012).

In eastern Washington foliar infections on sweet cherry start 4-6 weeks after bud break. Powdery mildew incidence and severity increase throughout the months of May and June. Foliar infections of powdery mildew spread to cherry fruits and there have been reports that entire fruit lots were rejected even with low incidence (Grove 1991). Sprague in 1957 contended that powdery mildew perennates as mycelium in dormant buds of sweet cherry and Choke cherry (*Prunus virginiana*); but Grove & Boal 1991 have demonstrated that *P. clandestina* (now *prunicola*) perennates as chasmothecia and acts as overwintering inoculum for the next season epidemics. It was not demonstrated whether the powdery mildew from chokecherry is the same as the one on sweet cherry and the powdery mildew from chokecherry could cause infections in sweet cherry or not.

Literature reviews have indicated that *Podosphaera oxycanthae* is the causal agent of the chokecherry powdery mildew (Lamey, H.A., Stack R. W 1991). The same pathogen has also been mentioned as a powdery mildew pathogen of other *Prunus* hosts. *Podosphaera tridactyla* had also been recently reported as powdery mildew fungus infecting the genus *Prunus* in most parts of the world including North America (Braun, U., Cook, R.T.A., 2012). It could be conceived that cherry trees might be co-infected with both *P. tridactyla* and *P. prunicola*; as there are reports of different powdery mildew species co-infecting the same host plant. For instance, in central Washington *Leveillula taurica* and *Golovinomyces orontii* were found co-infecting the leaves of potato (Glawe et al. 2004).

In Yakima Valley region, it was observed that chokecherry trees get powdery mildew infection in mid April (study conducted in the year 2012-2016), with in 20 days sweet cherry orchard located close to the chokecherry trees also gets powdery mildew infection; with in 20-25 days the remaining sweet cherry orchards also get the powdery mildew infection. The current study was carried out to determine whether the powdery mildew pathogen that infects *Prunus virginiana* is same as the one that infects *Prunus avium*, and to determine the evolutionary relationship of these two pathogens. The second objective of this study was to determine the species of *Podosphaera* infecting sweet cherries in Yakima valley region of Washington State.

Materials and methods

Sample collections

Two chokecherry (*Prunus virginiana* L.var. *melanocarpa*) powdery mildew fungal isolates were collected from chokecherry trees that are in close proximity (0.6 miles away) to an organic cherry orchard. Ten isolates of sweet cherry powdery mildew were collected from different cherry growing regions of Washington and 5 isolates were collected from experimental orchards located at the Washington State University Irrigated Agriculture Research and Extension Center (IAREC), Prosser, WA (Table 1). (Table 1 shows the powdery mildew isolate collection locations). Cherry foliage and other host foliage that were heavily infested with powdery mildew were placed in 1-liter Ziploc bags. Bags were labeled with the date and collection location and transported to the lab by keeping them on ice in a cooler (Coleman 48-quart cooler).

Morphological study

Symptomatic leaves of chokecherry and sweet cherry were examined using a stereomicroscope (Leica Microsystms Inc Buffalo Grove, IL, USA). The mycelium, conidia and chasmothecia were transferred onto a microscopic slide containing a drop of water, which was then covered using a coverslip and examined under Zeiss-Axio observer.Z1 at 100 X to 1000 X (Carl Zeiss Microscopy LLC, Thornwood, NY, USA). Morphological features evaluated included diameter of chasmothecia, number of ascospores per asci, length and width of chasmothecial appendages, arrangement of appendages and germination of the conidia. At least 50 measurements for each character were taken per specimen for comparison with the descriptions of the genus *Podosphaera*.

DNA isolation

Cherry leaves heavily infested with powdery mildew fungi were choppped in pieces and placed in 50 ml falcon tubes with 15ml of 0.001% Tween20 solution (Sigma-Aldrich, St. Louis, MO) and the tubes were subjected to vortexing for 1 minute, after which the leaf fragments were removed. The tubes were subjected to centrifugation at 5,000 rpm for 2 minutes. The supernatant was discarded and the white powdery mildew pellet was collected into bead solution tubes of Power Clean soil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA) for DNA extraction by following the manufacturer's protocol.

Apple, peach, rose, hop and peony powdery mildews were collected using a sterile pipette tip under a stereomicroscope, and single conidial chains were directly placed into PCR tubes containing the master mix. This ensured that the samples were not contaminated with other powdery mildew isolates, as the powdery mildew isolates were collected from various host plants in close proximity to each other. For negative control, a sterile pipette tip was immersed in PCR master mix.

PCR amplification with ITS+LSU region

Primer ITS1 F (CTTGGTCATTTAGAGGAAGTAA, Gardes & Bruns 1993) and primer TW 14 (GCTATCCTGAGGGAAACTTC, Hamby et al 1998) that amplifies the first internal transcribed spacer (ITS1) region, 5.8S rRNA gene, the second ITS (ITS2), which amplifies the 5' end of the 28 S large subunit of ribosomal RNA including the D1 and D2 regions were used. PCR reactions were conducted in 25 µl volumes for each sample containing 1X PCR standard buffer (New England Biolabs, Ipswich, MA),

200µM dNTPs (New England Biolabs), 20µM of each primer and 2.5 units Taq polymerase; 1 µl of undiluted DNA was used as template.

Birometra T Gradient thermo cycler (Whatman Biometra GmbH, Gottingen, Germany) was used for PCR amplifications. The reactions were cycled between 94°C (initial denaturation) for 4 minutes, 94°C (DNA denaturation) for 45 seconds, 54°C (oligonucleotide primer annealing) for 45 sec, and 72°C (Taq polymerase extension) for 1 min, and final extension of 72°C for 10 min. DNA denaturation, annealing and extension were repeated for 35 cycles. A negative control without template DNA also was included in each set of PCR reactions. The amplified fragments were visualized by 1.2 % agarose gel electrophoresis; the gels were stained with gel red and the DNA bands were visualized under a UV- transilluminator (Biorad Universal Hood, Bio-Rad Laboratories).

Amplified DNA fragments were purified from PCR products using QIAquick PCR purification kit (Qiagen GmbH) and cloned into plasmid pCR2.1 TOPO (Invitrogen Corp., Carlsbad, CA) and transformed into One Shot Top 10 chemically competent cells by following the manufacturer's protocol. Between 60-80 µl of each transformation mixture was spread and grown on Luria- Bertani (LB) agar plates containing 50 µg/ml of X- Gal (5- bromo-4-chloro-3 indolyl-b-D-galactopyranoside) and 50µg/ ml of Kanamycin (Invitrogen Corp., Carlsbad, CA) for selection of white colonies from blue colonies. Positive colonies were detected by colony PCR and were grown in 2 ml of LB broth (10 g of Tryptone, 5 g of yeast extract and 10 g of NaCl) containing 50 µg/ml kanamycin at 37°C in a horizontal shaker overnight. Plasmids were isolated using the QIAprep Spin Miniprep Kit. The plasmid concentration was determined using Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA).

DNA sequencing

Plasmids were sequenced at ELIM Biopharm (Hayward, CA, USA) and Retrogen (Retrogen Inc, San Diego, CA) using M13F and M13R primers. Internal primers ITS4 (reverse), ITS5 (forward), LROR, LR3, TW14 were also used for sequencing. Nucleotide sequences were assembled and edited using Vector NTI Advance 11software (Invitrogen Corp., Carlsbad, CA). Sequences were aligned against sequences in GenBank database using BLASTn search at http://www.ncbi.nlm.nim.gov/BLAST to identify similar sequences available in the database. All the edited sequences were submitted to the GenBank and the accession numbers of the powdery mildew isolates are listed in Table: 3. To compare the ITS region of *P. prunicola* and *P. tridactyla* with other *P. clandestina* and *P. tridactyla* isolates, accessions were chosen based on a combination of published studies and BLAST searches. (The ITS sequence isolates and their accession numbers are listed in the Table 7). For phylogenetic study of the ITS-LSU region, multiple sequence alignments of sequences from the ITS-LSU region of different powdery mildew isolates were performed using ClustalW in BioEdit (Hall, T.A., 1999) with default parameters. *Erysiphe* spp. isolate sequences were used as outgroup. (Figure7). For the ITS region, LSU sequence nucleotides were removed to obtain only sequences for the ITS region (Figure 8). These were aligned with sequences from GenBank using ClustalW in BioEdit (Hall, T.A., 1999). Neighbor-joining (NJ) phylogenetic trees were constructed using the MEGA5 (Tamura et al 2011) software package for ITS+LSU and ITS sequences. Bootstrap values were determined from 500 replicates to determine robustness of observed phylogenetic grouping, and branches with <70% bootstrap support were

collapsed. Sequence similarities were determined using Sequence Identity Matrix in BioEdit sequence alignment editor (Hall, T.A., 1999).

Greenhouse cultivation of chokecherry seedlings

Fruits of (*Prunus virginiana* var. *melanocarpa*) were collected from chokecherry trees in the fall of 2013. The flesh (pericarp) was removed using a pit cracker and the seed was stored in vermiculite treated with Captan spray (Captan; N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide 80 WDG, 4.5 mg per 3.785 liters of water manufactured for Arysta LifeScience North America corporation Cary, NC). The seeds were stratified at 4 °C for two months and then kept at room temperature to allow germination. In February 2014 germinated seedlings were transplanted to 6 cm diameter, 10 cm deep pots filled with WSU soil mix and kept in green house. The leaves of the plants were used for cross inoculation purposes.

Cross inoculation of powdery mildew conidia to test host compatibility

Inoculum preparation

Sweet cherry leaves (cv. Bing) heavily infected with powdery mildew fungi were selected from WSU-IAREC experimental orchard. Leaves were chopped into small pieces using sterile razor blade. The leaf pieces were transferred to a sterile 50 ml falcon tube with 10ml of 0.001% Tween 20, and vortexed for few seconds. Spore concentration of the resulting solution was determined using heamocytometer and adjusted to 1000 conidia/mL. Powdery mildew conidia from chokecherry leaves were isolated and the spore suspension was prepared as mentioned above.

Leaf disc preparation

Fully expanded young leaves from plants free of powdery mildew were excised from green house grown sweet cherry (cv. Bing) and chokecherry plants. Leaves were surface sterilized by immersing in 0.086% of calcium hypochlorite solution for 1 min, rinsed 3 times in sterile distilled water, and blotted dry on a sterile paper towel. Using cork borer #6 (11mm diameter) leaf discs were excised. About six discs were excised per leaf. The leaf discs were transferred to 6 different plates. Each plate contained 3 leaf discs that came from 3 different leaves and placed on the plate with the abaxial side facing up.

Cross inoculation on leaf discs

The cross inoculation test was conducted 3 times with 2 isolates of choke cherry powdery mildew pathogen and 4 different isolates of sweet cherry powdery mildew pathogen. First fully opened young leaves were excised from sweet cherry cv. sweet heart and chokecherry var. *Prunus virginiana var. melanocarpa* trees and leaf discs were prepared as stated earlier, conidial suspension of sweet cherry powdery mildew was pipetted on to chokecherry leaf disc and chokecherry powdery mildew conidial suspension was pipetted on to sweet cherry leaf discs. The experiment was repeated 3 times in early May of the years 2014 and 2015. The plates were incubated at 21°C with 12 hr light and dark cycles. The leaf discs were observed daily for powdery mildew colony development using a dissecting microscope.

Field experiment

Three potted *Prunus avium* cv. Bing trees (that were not treated with fungicide) were placed besides wild chokecherry bushes (located on Richards road, Prosser). Each *P. avium* plant was enclosed in Nitex bag with a heavily infested chokecherry branch to exclude external inoculum. After 15 days, Bing plants were brought back to the

greenhouse and the leaves were observed for signs of powdery mildew following removal of the Nitex bag.

Green house experiment

Chokecherry seedlings were inoculated with sweet cherry powdery mildew conidia. One group of sweet cherry seedlings were inoculated with sweet cherry powdery mildew conidia and second group of sweet cherry seedlings were inoculated with choke cherry powdery mildew conidia using a paintbrush; the plants were monitored for powdery mildew signs.

Results

Morphological study

Sweet cherry powdery mildew fungus

Sweet cherry powdery mildew fungus: Powdery mildew on seedling leaves manifested hyaline mycelium in scattered patches. Mycelium on leaves was amphigenous, epiphyllous, septate and thin walled with branched hyphae. Conidiophores arising from the upper surface of the hyphae had straight and cylindrical foot cells bearing chains of conidia (catenesecent) in basipetal manner. Conidia were ellipsoidal in shape and contained fibrosin bodies. The length of the conidia ranged between 22-28.9 μM long (average length = 26 μM) with a diameter of 11-19 μM (average width = 17.4 μM); showing terminal to lateral germ tubes (figure 6). Chasmothecia were scattered to gregarious, with a diameter ranging from 95-100 (average diameter 115 μM), appendages arising equatorially, and apices 2-3 times tightly dichotomously branched. Peridium cells were distinct, irregularly polygonal to round. Chasmothecium contained a single ascus

with 8 ascospores, which were ellipsoid- ovoid in shape, occasionally reniform, 20-28 μm long.

Chokecherry powdery mildew fungus

Mycelium was amphigenous, with branched, septate, thin walled hyphae. Conidiophores arising from the upper surface of hyphae, foot cells were straight and cylindrical bearing chains of conidia (catenesecent) in basipetal manner. Conidia ellipsoid in shape and have shown irregular fibrosin bodies, 21-30 μM (average length was 27 μM) X 10-19 μM(average width was 15.45 μM), and germ tubes were terminal to lateral. Chasmothecia scattered to greagaious, with a diameter ranging 95- 100-115 μm. Chasmothecial appendage apices were straight initially and become dichotomously branched during later stages. The length of the appendages ranges from 150-290 μm. Appendages were few in number, stiff and straight, arising in the upper half of the chasmothecium, almost fasciculate. Chasmothecium contained a single ascus containing 8 ascospores, which were ellipsoid- ovoid in shape.

rDNA sequences and phylogenetic study

Successful PCR amplifications were obtained with all isolates including the direct use of conidia in PCR master mix. Amplicon sizes ranged from 1400-1900 bp. When the rDNA sequence of the *P. prunicola, P. tridactyla, P. leucotricha, P. macularis, P. pannosa* were used in BLAST searches, the sequences in GenBank with highest similarity were *Fibroidium* spp. MUMH (AB 525946.1), *Fibroidium* spp. MUMH 4937 (AB 525945.1) and *Podosphaera clandestina* (AB 525927.1) with 99% identity. In Neighbor-Joining phylogenetic tree, all sweet cherry powdery mildew isolates formed a single clade; this was strongly supported by the bootstrap value of 93, whereas chokecherry powdery

mildew isolates were in a different clade. The genus *Erysiphe* which was used as an outgroup formed a separate clade (figure 7). All the sequences used in this study were submitted to GenBank and the accession numbers are shown in Table: ‘7

Sequence identity matrix

P. tridactyla rDNA ITS +LSU nucleotide sequences were 99 % to 99.2% identical to *P. prunicloa* rDNA ITS +LSU nucleotide sequences. The nucleotide sequence identity among *P.prunicola* isolates range from 99.5 % to 100 %. *P. tridactyla* rDNA ITS +LSU nucleotide sequences were 95% to 99% identical to other *Podosphaera* spp. isolates from various hosts. The nucleotide sequence identity among *Podosphaera tridactyla* and genus *Erysiphe* ranges from 88.9 to 89.3%. The nucleotide sequence identity among the genus *Erysiphe* isolates range from 88 % to 96.4%.

Cross inoculation

Leaf disc cross inoculation

None of the sweet cherry leaf discs showed powdery mildew signs when inoculated with chokecherry powdery mildew conidia. In the same manner none of the chokecherry leaf discs developed powdery mildew signs when inoculated with sweet cherry powdery mildew conidia. Whereas chokecherry leaves inoculated with powdery mildew conidia that infects one type of choke cherry (Prunus virginiana var melanocarpa) also showed powdery mildew signs when inoculated on another variety of chokecherry (Prunus virginiana var virginiana). The sweet cherry cv. Bing plants that were left along with chokecherry tree, did not show signs of powdery mildew even when the plants were left for extended periods of time beneath infested chokecherry bushes. Experiments that were conducted in the Green house showed the similar results; chokecherry seedlings

showed powdery mildew signs when inoculated with choke cherry powdery mildew conidia whereas sweet cherry seedlings (cv. Bing) did not show the signs of powdery mildew when inoculated with chokecherry powdery mildew conidia.

Discussion

Occasionally chokecherry trees happen to occur close to commercially grown sweet cherry orchards. This study was conducted to determine whether the Erysiphaceous fungus that infects *Prunus virginiana* var. *melanocarpa* is same as the one that infects *Prunus avium*. Anamorphic features of the two fungi were similar. The teleomorph features that are useful in species determination include the size and shape of chasmothecia; number, size and morphology of chasmothecial appendages; ascus number and shape; ascospore size and shape (Braun, U., Cook, R.T.A., Inman, A.J., Shin, H-D. 2002) and these characteristics were used to determine the species of chokecherry and sweet cherry powdery mildew fungi in this study. Generally chasmothecial appendages are used to distinguish genera are now also being used to distinguish species (Braun,U., Takamatsu, S., 2000). The teleomorphic stage of chokecherry powdery mildew fungus showed two major differences from that of sweet cherry powdery mildew fungus (Figure 1). The chasmothecial appendages of choke cherry powdery mildew fungus were fasciculate; which is a characteristic feature of *P. tridactyla* (Braun, U., Cook, R.T.A., 2012). In case of sweet cherry powdery mildew fungi, the chasmothecial appendages were arranged around the equatorial region, the apices were branched dichotomously 3-4 times; these morphological descriptions were in close agreement with the description of *Podosphaera prunicola* (Braun, U., Cook, R.T.A., 2012). 2. Most of the chasmothecial appendage apices in chokecherry powdery mildew did not show any dichotomous

branching, indicating that it is not same as the fungus that infects sweet cherry. The majority of the *Podosphaera* species parasitic to *Prunus* have been classified as *P. tridactyla* (Takamatsu et al 2010). This study also confirmed that the powdery mildew infecting *P. virginiana* is *P. tridactyla.* *P. tridactyla* complex was found to be paraphyletic with *P. xanthii* and *P. fusca* , (Figure 8) this finding agrees with that of Cunnington et al 2005. *P. tridactyla* has high lever of genetic variation and could be divided into several groups based on host specialization. (Cunnington et al 2005).

Neighbor joining analysis of ITS + LSU region revealed that these *P.trydactla* from chokecherry and *P. prunicola* from sweet cherry are closely related but they did not share same clade (Figure 1). *P. tridactyla* would have specialized to the respective hosts along with the genetic divergence of *Prunus* hosts(Takamatsu et al 2010). The present study also confirmed that the powdery mildew pathogens of different *Prunus* (sweet cherry, choke cherry and peach) hosts diverged differently and formed separate clades during neighbor joining analysis of ITS +LSU region. Interestingly the *P. macularis* (hop powdery mildew pathogen, the host belongs to the family *Cannabaceae*) clade lying in between the *P. tridactyla* and *P. pannosa* clades, which belongs to the family *Rosaceae*, and this finding is similar to the findings of Takamatsu et al (2010).

Evolutionary relationships among powdery mildew fungi have been determined using ITS sequence of rDNA. A good correlation was observed between the morphological classification system for the powdery mildews (Cook 1997) and ITS sequence based classification developed by Saenz and Taylor in 1999. Neighbor-joining analysis of ITS region was carried out to compare evolutionary relationship of the chokecherry powdery mildew fungus with other *P. tridactyla* isolates infecting various

Prunus hosts (figure 2). Sequences of *P. tridactyla* from this study were distantly related to the *P. tridactyla* isolates that infect bird cherry, plum cherry, black cherry and other wild cherries. *P.tridactyla* isolate clusters with *P.clandestina* and *P. prunicola* isolate in a separate clade from all other *P. tridactyla* isolates on other *Prunus* hosts. This placement would appear counter to phylogenetic analyses of *Podosphaera* species by Takamatsu and co workers (2010) and Cunnington (2005). It is unexpected to see *P. tridactyla* isolate from Korea that infects sweet cherry formed a separate clade from other *Podosphaera* isolates from North America. *P tridactyla* from Yakima valley region was shown to be more closely related to the other *P. prunicola/ clandestina* isolates of Yakima valley region and North American isolates. *P. tridactyla* from chokecherry was next to *P.prunicola* in both ITS+ LSU and ITS region phylogenetic trees. The non-availability of *Podosphaera* species isolates from chokecherry in GenBank and the non-availability of powdery mildew isolates from chokecherry in different locations of WA State or elsewhere in the USA, is a limiting factor for understanding the detailed relationship of *P. prunicola* and *P. tridactyla* in this present study.

Some criteria for speciation that can be taken into account are host range, specialization and geographical peculiarities (Braun, U., 1995). Cross inoculations that were carried out in growth chamber, field and lab studies have shown that the chokecherry powdery mildew conidia could not infect sweet cherry; *P. tridactyla* host range is restricted to choke cherry varieties only. In a similar way the powdery mildew conidia from the sweet cherry did not cause infection on chokecherry even after inoculating with 3 different isolates of *P. prunicola*. This indicates that these pathogens are restricted to their respective hosts. Hirata and Takamatsu (2001) proposed that

restricted host range may cause niche separation of powdery mildews and can generate genetic diversity in the fungi. In this study also it was found that *P. prunicola* isolates rDNA ITS+ LSU sequences were highly identical, whereas the sequence identity among *P. prunicola* and *P. tridactyla* was less identical. In *P.tridactyla* isolate there were 8 nucleotide substitutions out of 100 nucleotides in the ITS+LSU region of rDNA compared to *P. prunicola* isolates. This study indicates that the genus *Podosphaera* isolates from diverse *Prunus* hosts show less sequence diversity in the rDNA ITS+LSU region compared to the genus *Erysiphe* isolates. Based on all these evidences it was determined that chokecherry and sweet cherry are infected by two distinct host specific *Podosphaera* species.

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Table 1: List of sweet cherry powdery mildew isolates from cherry growing regions in Washington State and other powdery mildew isolates that infect different hosts in these regions. All *P.prunicola* isolates that were collected in Prosser were from different orchards located 10 km radius from each other.

Powdery mildew	**Host genus and species**	**Origin**
Podosphaera prunicola	*Prunus avium*	Pasco, WA
P. prunicola	*Prunus avium*	Wenatchee, WA
P. prunicola	*Prunus avium*	Quincy, WA
P. prunicola	*Prunus avium*	Quincy, WA
P. prunicola	*Prunus avium*	Prosser WA
P. prunicola	*Prunus avium*	Prosser, WA
P. prunicola	*Prunus avium*	Prosser WA
P. prunicola	*Prunus avium*	Benton city, WA
P. prunicola	*Prunus avium*	Benton city WA
P prunicola	*Prunus avium*	Mattawa, WA
P.prunicola	*Prunus avium*	Prosser, WA
P. tridactyla	*P. virginiana var. melanocarpa*	Prosser, green house, WA
P. tridactyla	*P. virginiana var. melanocarpa*	Prosser, WA
P. leucotricha	*Malus domestica*	Prosser, WA
P. macularis	*Humulus lupulus*	Prosser, WA
P. pannosa	*Rosa gallica*	Prosser, WA
P. pannosa	*Rosa gallica*	Prosser WA
P. pannosa.	*Prunus persica*	Prosser, WA
P. xanthii	*Cucurbita pepo var. Fastigata*	Prosser, WA
P. fusca	*Taraxacum officinale*	Pasco, WA
Erysiphe graminis	*Poa species*	Prosser, WA
E. necator	*Vitis vinifera*	Prosser, WA
E. convovlvuli	*Convolvulus arvensis*	Prosser, WA
E. paeoniae	*Paeonia lactiflora*	Prosser, WA
P. fusca.	*Taraxacum*	Pasco, WA
E. cichoracearum	*Cirsium arvense*	Prosser WA
E.polygoni	*Polygonum erectum*	Prosser WA

Table 2: Sequence identity matrix: comparison of *P. prunicola* isolates ITS+LSU sequences with that of *P. tridactyla*. The ITS+ LSU nucleotide sequences of various *P. prunicola* share 99.5 % to 100 percent similarity, whereas the similarity between *P. prunicola* and *P. tridactyla* was 99.0 to 99.2

Seq	*P. tridactyla*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*	*P.prunicola*
P. tridactyla	ID													
P.prunicola	0.992	ID												
P.prunicola	0.992	1	ID											
P.prunicola	0.992	1	1	ID										
P.prunicola	0.99	0.998	0.998	0.998	ID									
P.prunicola	0.99	0.998	0.998	0.998	0.997	ID								
P.prunicola	0.99	0.998	0.998	0.998	0.996	0.996	ID							
P.prunicola	0.99	0.997	0.997	0.997	0.996	0.996	0.995	ID						
P.prunicola	0.992	1	1	1	0.998	0.998	0.998	0.997	ID					
P.prunicola	0.992	1	1	1	0.998	0.998	0.998	0.997	1	ID				
P.prunicola	0.991	0.999	0.999	0.999	0.998	0.998	0.997	0.996	0.999	0.999	ID			
P.prunicola	0.992	0.998	0.998	0.998	0.997	0.997	0.996	0.997	0.998	0.998	0.998	ID		
P.Prunicola	0.99	0.998	0.998	0.998	0.996	0.996	0.996	0.995	0.998	0.998	0.997	0.996	ID	
P.prunicola	0.992	1	1	1	0.998	0.998	0.998	0.997	1	1	0.999	0.998	0.998	ID

Table: 3 Sequence Identity matrix, comparison of rDNA ITS+ LSU nucleotide sequences of *P.tridactyla* with other powdery mildews in the genus *Podosphaera*. The similarity between *P.tridactyla* and other *Podosphaera* spp. is 95.7 % to 99%, indicating that the ITS+ LSU region among *Podosphaera* spp. isolates is highly conserved

Seq->	*P.tridactyla*	*P. pannosa*	*P. xanthii*	*P. fusca*	*P. leucotricha*	*P. macularis*	*P. pannosa*
P.tridactyla	ID						
P. pannosa	0.990	ID					
P. xanthii	0.957	0.959	ID				
P. fusca	0.959	0.959	0.988	ID			
P. leucotricha	0.965	0.965	0.945	0.947	ID		
P. macularis	0.988	0.987	0.955	0.957	0.964	ID	
P. pannosa	0.989	0.999	0.958	0.958	0.965	0.986	ID

Table 4: Sequence Identity matrix, comparison of rDNA ITS+ LSU nucleotide sequences of different *Erysiphe* species and *Podosphaera tridactyla*. The ITS+ LSU nucleotide sequence similarity among various *Erysiphe* spp. range from 88.4- 94.5% indicating that this region is more diverse in the genus *Erysiphe*

Seq->	*P.tridactyla*	*E. necator*	*E. graminis*	*E. paeoni*	*E. convolvuli*	*E. cichoracearum*
P.tridactyla	ID					
E. necator	0.893	ID				
E. graminis	0.891	0.884	ID			
Erysiphe paeoni	0.889	0.945	0.886	ID		
E. convolvuli	0.891	0.941	0.880	0.964	ID	
E.cichoracearum	0.893	0.907	0.900	0.912	0.906	ID

Table 5: The name, origin and sequence accession number for isolates of ITS region obtained from GenBank and used in phylogenetic analysis

Species	Host Plant (common name in parenthesis)	Country of origin	Sequence accession	Reference
Podosphaera pannosa	*Rosa maltiflora*	Japan	AB525939	Takamatsu et al 2010
P.tridactyla	*Prunus armeniaca* (Apricot)	Australia	AY833657	Cunnington et al 2005
P.tridactyla	*P. cerasifera* (plum cherry)	Australia	AY833656	Cunnington et al 2005
P.tridactyla	*P. cerasifera* (plum cherry)	Australia	AY833658	Cunnington et al 2006
P.tridactyla	*Prunus* sp.	Kongung, Korea	AY833659	Cunnington et al 2007
P.tridactyla	*P. persica* (peach)	Australia	AY833651	Cunnington et al 2008
P.tridactyla	*P. padus* (bird cherry)	Kongung, Korea	AY833652	Cunnington et al 2009
P.tridactyla	*P. laurocerasus* (cherry laurel)	Switzerland	AY833654	Cunnington et al 2010
P.tridactyla	*P. lusitanica* (cherry bay)	Switzerland	AY833655	Cunnington et al 2011
P. clandestina	*Prunus* sp. (black cherry)	Mexico	KJ476999	Santiago et al 2014
P. clandestina	*P. avium* (sweet cherry)	USA	AF011316	Saenz et al 1999
P.tridactyla	*P. avium* (sweet cherry)	Korea	KP 710960	Choi et al 2015

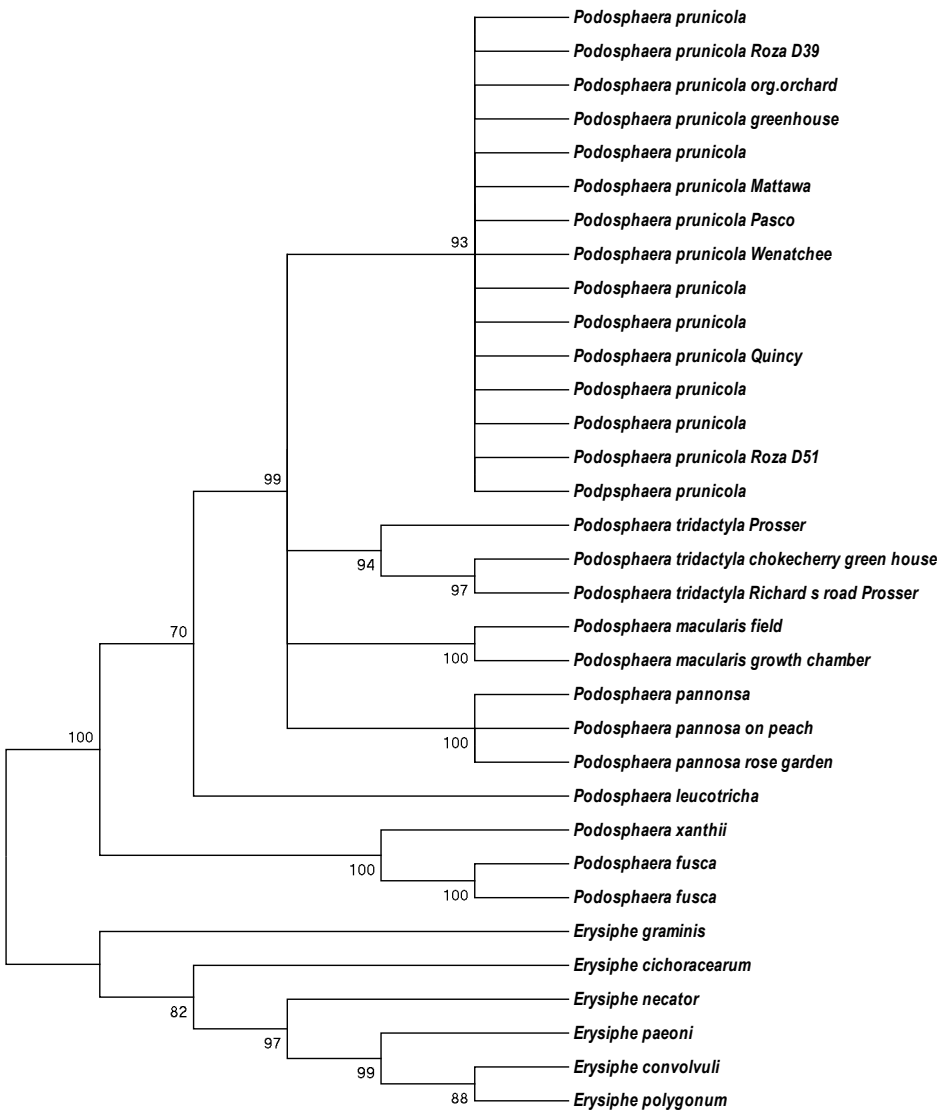


Figure 1: Neighbor-joining analysis of Internal transcribed spacer and large subunit of ribosomal DNA sequences showing close evolutionary relationship of *Podosphaera prunicola* and *Podosphaera tridactyla* with other powdery mildew sequences from Yakima valley, (all the isolates were collected for this study. Trees were constructed by neighbor- joining algorithm with the Kimura two parameter model implemented by MEGA 7. Bootstrap values are indicated at the branch nodes.

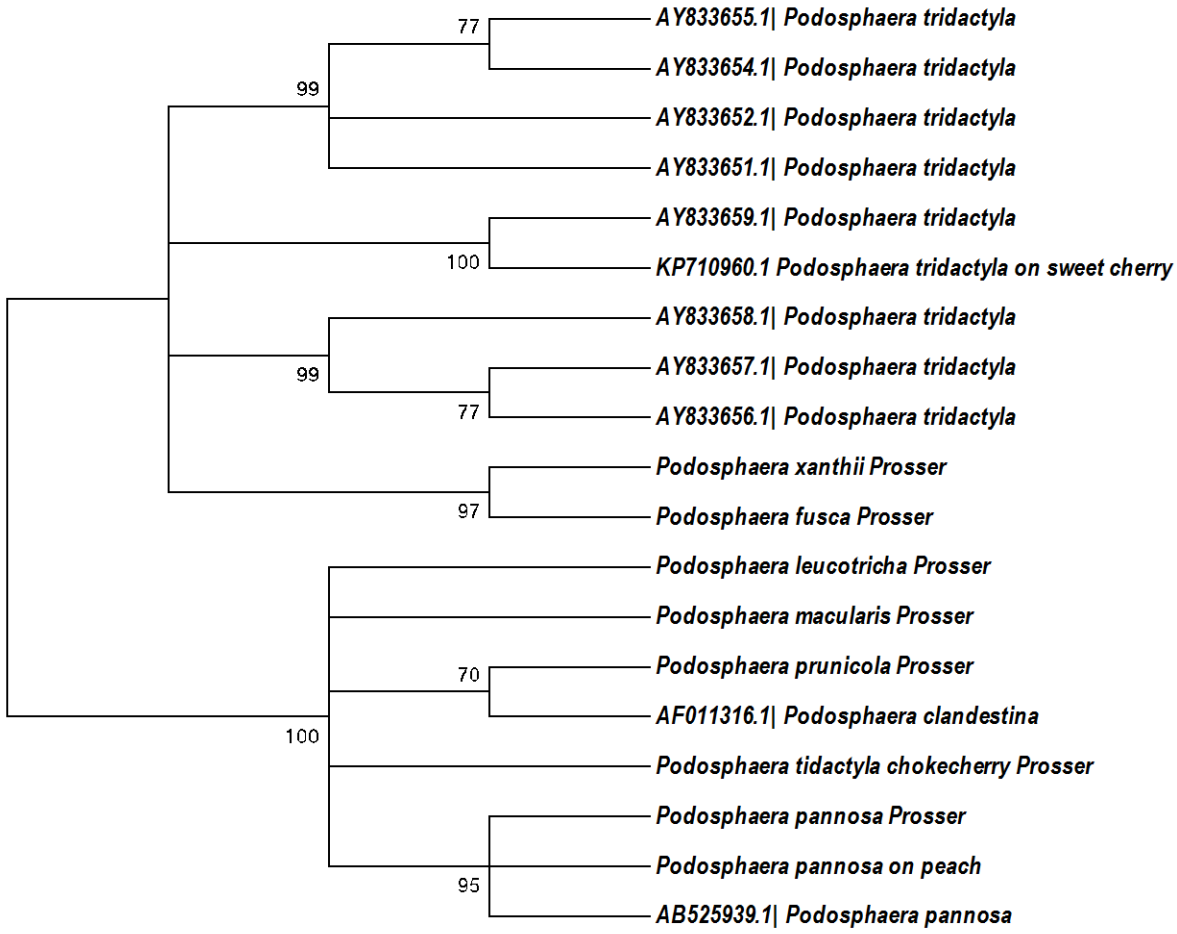


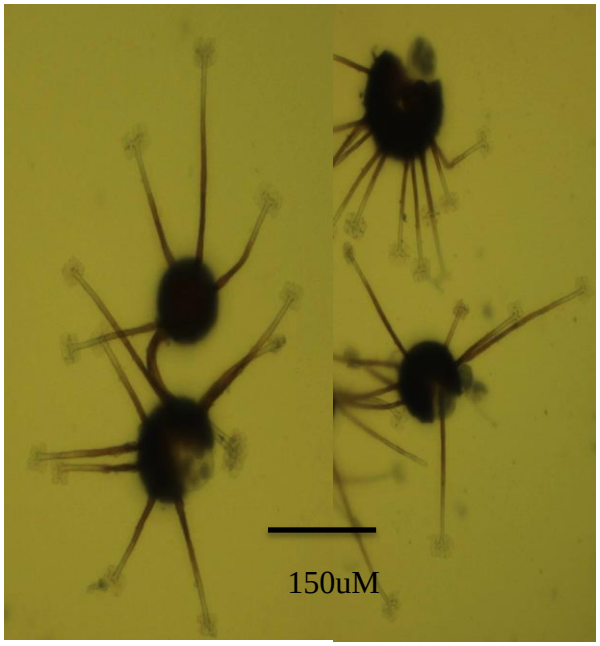
Figure 2: Neighbor-joining analysis of ribosomal DNA ITS sequences showing close evolutionary relationship of *Podosphaera prunicola* and *Podosphaera tridactyla* with other powdery mildew sequences from GenBank. Trees were constructed by neighbor-joining algorithm with the Kimura two parameter model implemented by MEGA 7. Bootstrap values are indicated at the branch nodes, bold taxa indicate the sequences from this study.

Table 6: Pathogenicity tests conducted on sweet cherry and chokecherry leaf discs with the isolates of *P.prunicola* and *P.tridactyla*; sweet cherry leaves when inoculated with *P.prunicola* isolates showed powdery mildew signs after 7 DPI, in the similar way chokecherry leaves (2 different varieties) when inoculated with *P.tridactyla* showed powdery mildew signs after 7 DPI. *P.prunicola* did not show powdery mildew signs when inoculated on chokecherry leaves, *P.tridactyla* conidia did not develop into powdery mildew colony when inoculated on to sweet cherry leaves.

Cross inoculation	*P.avium*	*P.virginiana Var.melanocarpa*	*P.virginiana var.virginiana*
P.prunicola	+	–	–
P.tridactyla	–	+	+

Table 7: List of powdery mildew isolates from Yakima Valley region (all from this work) and their GenBank accession number

Powdery mildew fungal sequence	GenBank Accession number
Podosphaera clandestina	KX826855
Podosphaera prunicola	KX842345
P.tridactyla	KX842346
P.fusca	KX842347
P.pannosa	KX842349
P.leucotricha	KX842350
P.xanthii	KX842351
P.pannosa	KX842352
Eryspihe peoniae	KX842353
E.cichoracearum	KX842354
E.convolvulii	KX842355
E.necator	KX842356
E.graminis	KX858799
P.macularis	KX858801
E. polygoni	KX826856
P.prunicola	KX858800



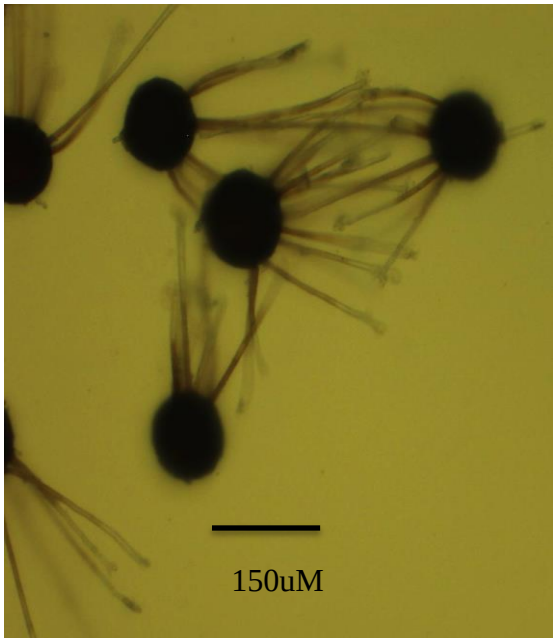
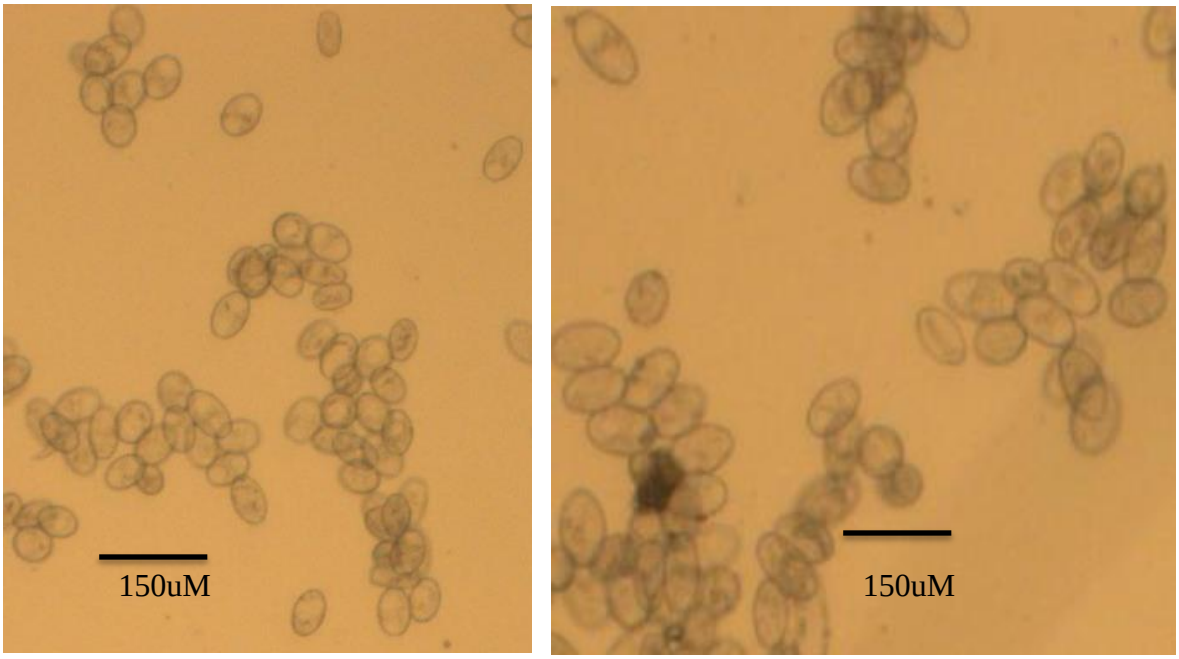


Figure 3a and 3b (left): *P. prunicola* chasmothecial appendages arranged around the equatorial region. Figure 3c(right): *P.tridactyla* with fasciculate appendages.



Figures 4a (left): Magnification 100 X, scale -150 μM. Conidia of *Podosphaera prunicola* ; Figure 4b (right) conidia of *Podosphaera tridactyla*.

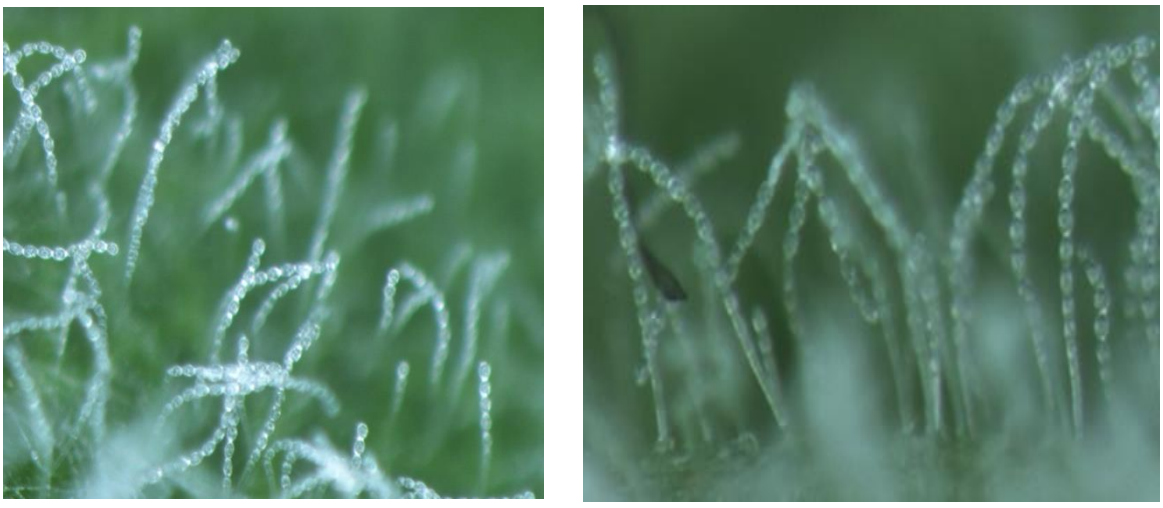
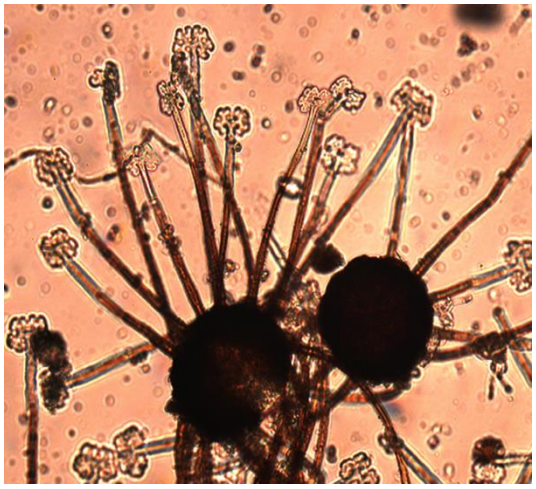


Figure 5a (left): *P. prunicola* conidial chains on sweet cherry leaf; Figure 5b (right): *P.tridactyla* conidial chains on choke cherry leaf



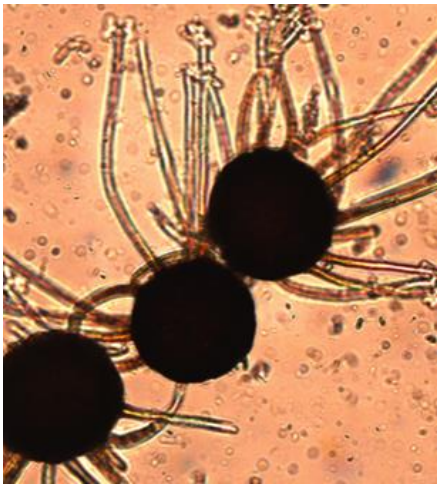


Figure 6a (left): *P. prunicola* chasmothecial appendages with more divergent branching pattern at their ends. Figure 6b (right): *P.tridactyla* with less or no divergent branching pattern at their ends

Figure 7a: cross inoculation experiment; sweet cherry leaf discs cv. Bing inoculated with *P. prunicola* conidia showing powdery mildew signs after 8 days of post inoculation (DPI)

Figure 7b:choke cherry leaf discs inoculated *P. prunicola* conidia showing no signs of powdery mildew after 8 (DPI).

Figure 7c: choke cherry leaf discs inoculated with *P. tridactyla* conidia conidia showing powdery mildew signs after 8 days of post inoculation

Figure 7d: sweet cherry leaf discs cv. Bing inoculated with *P. tridactyla* conidia showing no signs of powdery mildew after 8 (DPI).

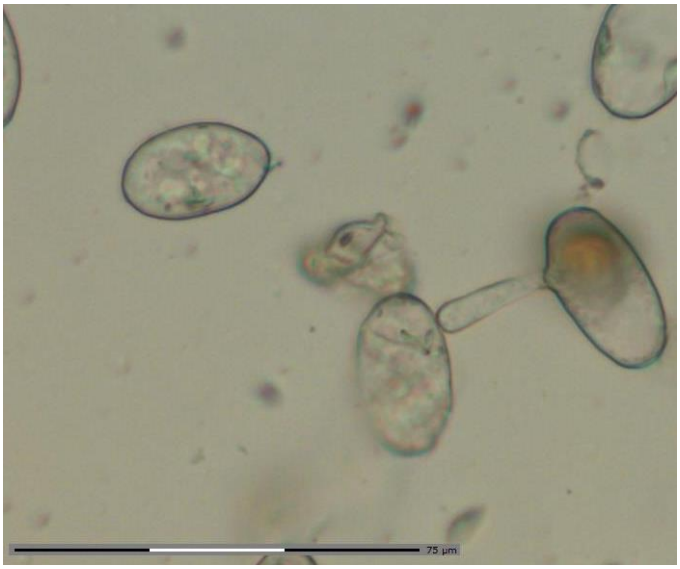


Figure 8: *P. prunicola* conidia showing sub terminal germ tube

CHAPTER THREE

EPIDEMIOLOGY OF POWDERY MILDEW IN WASHINGTON NURSERIES

Abstract

Sweet cherry nurseries are prevalent in the Columbia basin region of Washington State. Powdery mildew is the major disease problem in the sweet cherry nursery production. Rotary impaction air sampling devices were placed in the nursery for capturing conidia and/or ascospores of *P. prunicola* and DNA was isolated using a commercial kit. Quantitative real time PCR indicated that the airborne genetic material of *P. prunicola* was present from mid-May until mid-October. Symptoms and signs of powdery mildew first became apparent in the middle of June or early July, generally about one month after detection of pathogen DNA in air samples. Even though the rootstock carried viable chasmothecia, powdery mildew signs did not develop when infested plant material was incubated in the growth chambers temperatures ranging 10-23^{o}C and 80% relative humidity. Since the cherry powdery mildew inoculum was detected even before signs and symptoms development in the nursery it was assumed that the source of initial inoculum is most likely originating from the nearby sweet cherry orchard. There was a positive correlation among spore concentration, relative humidity and disease severity ($P<0.05$). Tree washings from the stored nursery stock have shown that the viability of the chasmothecia were high in the beginning of the year and went down to 0-2 % by June. Infested cherry trees that were planted in the nursery and green house did not develop the powdery mildew. Leaves collected from the previous year nursery floor and stored in a cage over winter had shown numerous viable chasmothecia

with viability ranging from 15 -10 % at the beginning of the year but the viability declined to 0- 2% by June. Filter paper containing the viable chasmothecia when placed on either attached cherry young leaves or detached cherry leaves did not cause any powdery mildew infections. The role of chasmothecia in the epidemiology of powdery mildew in cherry nurseries and distribution to growing areas via nursery stock remains unclear.

Introduction

Washington State nurseries rank number one for cherry tree production (personal communication with Bill Howell Northwest Nursery Improvement Institute). Sweet cherry powdery mildew is caused by *Podosphaera prunicola* is the major disease problem in the irrigated nurseries and orchards in Eastern Washington (Grove, G.G, Boal, R.J, 2000). The disease reduces the growth and vigor of the young trees in the nursery and orchard resulting in early defoliation; premature defoliation in turn results in reduced cold hardiness of the cherry tree (Webster, A.D and Looney, N.E 2006). The epidemiology of the disease in nurseries is poorly understood.

Once it was widely believed that many Erysiphaceous fungi (including those affecting grape and cherry) survived the winter exclusively as mycelium in dormant buds and the ascigerous stages were epidemiologically insignificant (Bulit and LaFon, 1978; Schnathrst, 1965; Yarwood, 1957; Johnson, 1983; Galloway, 1889; Sprague 1957). Chasmothecia of *P. prunicola* (previously known as *P.clandestina*) were demonstrated to be the only means of perennation in eastern Washington (Grove, G.G and Boal, R.J., 1991. It was demonstrated that the chasmothecia viability of *P. prunicola* in orchards

was high in mid- winter (early February; 55-90%) and decreased rapidly after bud break (5-33%) in mid- May (Grove, G.G and Boal, R.J., 1990).

Ascospores and conidia of the *P. clandesitna* (now *P. prunicola*) had been trapped from the air of the orchards using volumetric spore traps (Grove, G.G; Boal, R.J, 1991, Grove, G.G, 1998). Studies have shown positive correlation between aerial spore concentrations with higher the risk infection (Carisse et al, 2009a; Royle, 1978; Van der Heyden et al, 2013). Conventionally the airborne spores were collected using volumetric spore traps and the spores were identified by microscopic observation (Aylor, 1993; Carisse et al, 2009a Grove et al 1991, 1998 McCartney et al 1997). Advances in molecular biology have enabled researchers to detect and quantify pathogen populations using PCR assays (Vincelli and Tisserat, 2008; West et al 2008). PCR based diagnostic tools are more precise, accurate and less time consuming (Kong et al., 2003; Levesque, 2001). Rotary impaction spore traps were proven to be effective in collecting the grape powdery mildew spores; subsequent DNA extraction and PCR procedures have shown that these samplers were inexpensive and promising for the detection of *E. necator* conidia in the air (Falacy et al 2007). Within vineyards, the best sampling location is just above the crop canopy, where air is mixed from the crop and from external sources (Mahaffee et al 2014). The collection efficiency of various rotary impaction air samplers depends on the speed of rotating arms (West et al 2014). Understanding the disease epidemiology in nurseries can provide better management options. The purpose of this study was to develop a comprehensive understanding of powdery mildew of sweet cherries in nurseries.

Objectives

The objectives of the present study were 1) To determine the source of primary inoculum in the nurseries 2) To investigate the effects of meteorological factors on powdery mildew epidemics in cherry nurseries and 3) to investigate the perennation of *P. prunicola* in the dormant buds of cherry rootstock seedlings and 4) to determine the significance of chasmothecia on infested leaf detritus in the nursery.

Materials and methods

Nursery description

Commercial cherry nurseries (Van Well nursery) located 5 km west of Quincy were used for the epidemiological studies from 2012 to 2016. Plots comprised of sweet cherry cultivar 'Bing' were used throughout the study. Cherry plots were moved to different locations within a 24 km radius of Quincy each year.

Investigation of survival of chasmothecia on cherry seedlings

To evaluate the presence of mycelium or overwintering chasmothecia on the cherry seedlings, 2-year-old cherry seedlings were brought from cold storage to IAREC, Prosser in March and April during the years 2015 and 2016. Seedling washings were carried out by placing them on the top of a plastic sheet (that was folded to form a funnel shape), which was suspended above two nested (0.125mm and 0.074 mm) sieves (USA Standard Testing Sieves, Mentor, OH). The debris in the bottom sieve was backwashed and pushed to one side of the sieve and then collected in a 400 ml beaker by washing with about 100 ml of tap water. Samples were transferred back to the 400 ml beaker and the cylinder washed with 50 ml water that was then transferred to the beaker. A magnetic

stir bar was placed in beaker kept in suspension on a magna-stir apparatus. Ten milliliters of solution was measured quickly from the beaker (to avoid settling of the debris) and transferred to a Buchner funnel vacuum apparatus containing two 70 mm Whatman # 2 filter papers, which were transported to a dissection scope with forceps for observation. This procedure was repeated 4 times and the remaining solution was poured onto a single filter paper to collect remaining chasmothecia. All equipment was washed with warm water and soap and allowed air dry prior to processing subsequent samples. Five filter papers were visually examined with a dissection scope at 10 x, and the number of chasmothecia recorded.

Chasmothecia were then evaluated for viability by removing them from the filter papers with a fine probe and placing them in a drop of 1% NaCl salt solution on a microscope slide. A coverslip was added lightly depressed with a probe to burst the chasmothecia. . Chasmothecium / ascospore viability was determined using the methods of Pearson and Gadoury (1987), Grove and Boal (1991), and Cortesi et al (1997). The remaining 80 infested seedlings (with chasmothecia on the stem) were planted in 10 cm deep pots and the plants (20 plants per chamber) incubated in controlled-environment chambers that were set at 4 different temperatures, ranging 20- 28^{o}C Plants were watered daily; the relative humidity was set 80%. Using an atomizer, a fine mist was generated using an atomizer that creating conditions conducive for ascospore release.

Investigation of infection of dormant buds of cherry seedlings

Cherry seedlings were planted in 2 liter pots and placed in the growth chambers (Percival Scientific, City, state) at temperatures of 10°C to 23°C. Twenty plants were kept in each growth chamber. A 17W tube light was placed on the top of the growth

chamber (light intensity of 879 W/m^2, measured by Lux/FC light meter, MANNIX, DLM-204). Photoperiod was set for 12 hours. Relative humidity was monitored with sensors (HOBO data loggers, Onset Computer Corporation, Bourne, MA) and was positioned inside the growth chamber.

Investigation of chasmothecia and their viability on stored nursery stock

Cherry trees infested by *P. prunicola* in the nursery were tagged with flagging tape 1-3/16" non-adhesive plastic ribbon during October. Sixty trees were tagged which includes those treated with narrow-range petroleum oil and untreated control plots. Plants were dug in the month of November and placed in cold storage. Beginning in January of the following year, 10 trees were brought to the lab at Prosser IAREC and washed as described above. Each individual tree was cut above the grafted area and the upper part was taken and washed thoroughly using tap water. The remaining, lower part of the trees that had previously been cut for the washing experiment and chasmothecia viability assay were planted either in the greenhouse (when the daytime temperature was below 5°C) or in the field from the month of April during years 2013-2015. The trees were monitored for flag shoot development and for powdery mildew signs. In the year 2015 the plants were covered with 8 uM mesh Nitex bags to prevent contamination by external inoculum. Plants and potential attached chasmothecia were watered using a hose with water tank attached, to promote the release of ascospores.

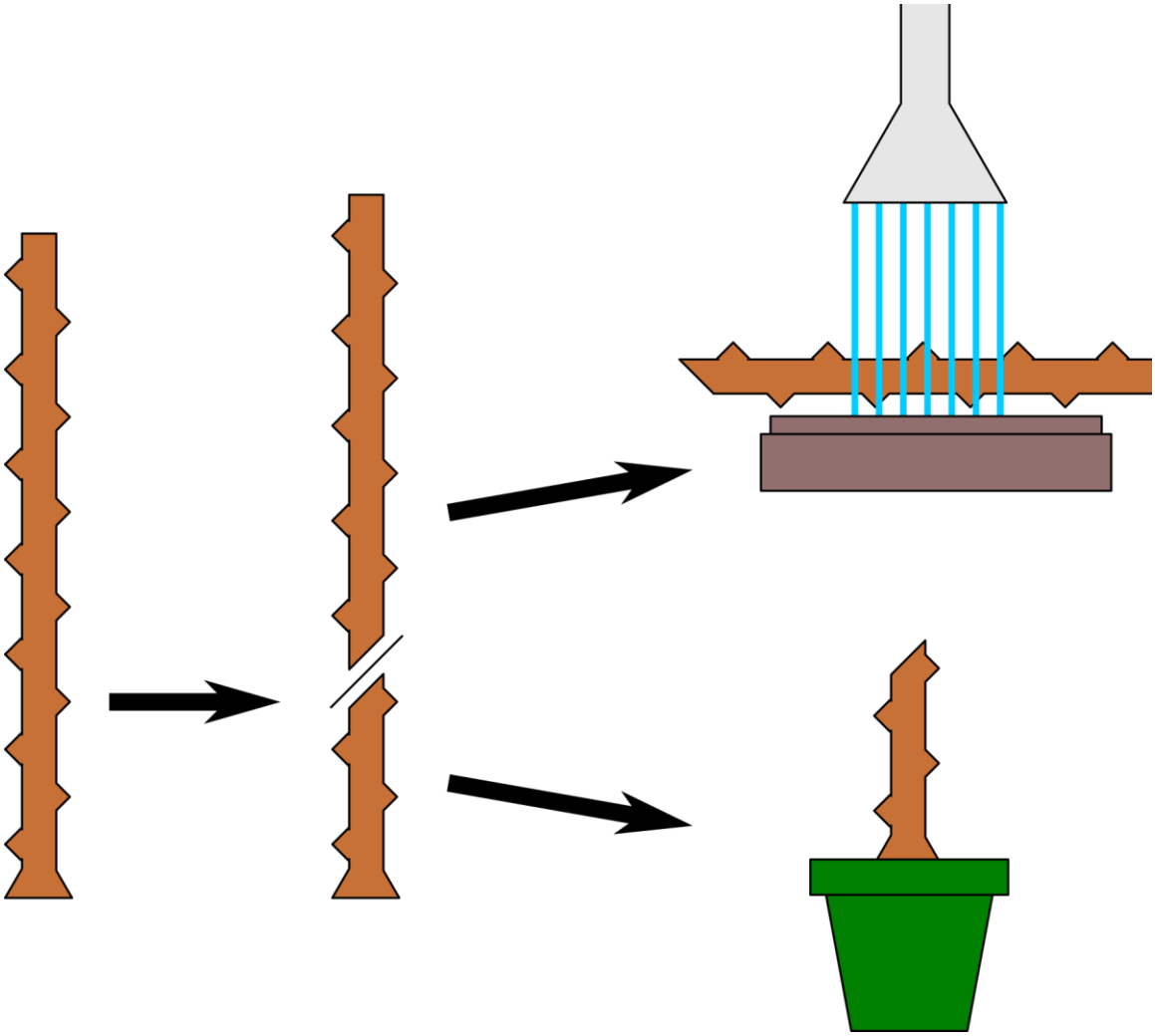


Figure 1: Tree washings and planting; figure generated using Inkscape version 0.92

Determining the significance of chasmothecia on nursery leaf debris

A 0.6m x 0.45 m x 0.304m (l x w x h) cage was constructed using a welded wire mesh and placed on the nursery floor in mid-October, cherry leaves were collected from high disease severity plots (that were not treated with fungicide) in the nursery**.**

Approximately 200 leaves were collected and placed in this cage; leaves along with the cage were placed in the nursery that was designated as the site of the following season's nursery. The leaves were thus subjected to the natural environmental conditions of the

nursery. Starting in December, about 20 leaves were collected at random from the cage and viability was determined using the aforementioned viability methods, and were carried out monthly until June of the subsequent year.

To evaluate the temporal infectivity of chasmothecia on infected senesced leaves, a leaf-grinding method was used. Samples (each of which contained 20 infected leaves) were collected monthly and transported to lab in paper bags. Dried leaves were crushed by hand and placed into 1 L Mason jars (Ball® Jar, City, State, Zip) and the blade unit from a blender (Oster ®Simple Blend ^{TM}) was attached to the Mason jar. Each batch was comminuted for 30 seconds with pulsed power in order to produce a uniform sample. The blade unit and jar were cleaned with compressed air and Kim-wipes after processing each sample. Sample weights were recorded and processed samples were stored in a refrigerator.

One gram of ground leaf material from each sample was transferred to the top of two nested sieves (USA Standard Testing Sieves, Mentor, OH) No. 120 (0.125mm) and 200 (0.074mm). Samples were washed twice for 30 seconds with a fan-shaped spray by squeezing the end of a 1 cm diameter plastic tube. Each sample was moved around the sieve to invert leaf fragments between washes. The debris in the bottom sieve was backwashed and pushed to one side of the sieve and then collected in a 400 ml beaker by washing with about 100 ml of water. This volume was measured in a graduated cylinder and adjusted to a final volume of 100 ml by adding water. Samples were transferred back to the 400 ml beaker and the cylinder was washed with 50 ml water that was then transferred to the beaker. A stir bar was placed in beaker and the beaker was placed on a magna-stir apparatus where debris was kept in suspension. Ten milliliters of solution

were measured immediately from the beaker (to avoid settling of the debris) and transferred to a Buchner funnel vacuum apparatus containing two 70 mm Whatman # 2 filter papers, which were transported to a dissection scope with forceps for observation. This procedure was repeated 4 times and the remaining solution was poured onto a single filter paper to collect remaining chasmothecia. All equipment was washed with warm water and soap and allowed air dry prior to processing subsequent samples. Five filter papers were visually examined at 10X and the number of chasmothecia recorded.

Figure 2: Attached leaf assay

Ascospore viability was determined using the methods of Pearson and Gadoury (1987), Grove and Boal (191), and Cortesi et al (1997). Filter paper disks containing chasmothecia was attached to the lid of Petri plate using silicone grease (Dow Corning high vacuum grease, Dow Corning Corporation, Auburn, MI) ; a single cherry leaf (cv. Bing) petiole was inserted into the Petri plate, with leaf blade facing the filter paper and the leaf and Petri plate were secured together using a rubber band.

Detached leaf assay

For the detached leaf assay, double Petri plates were used in which two Petri plates were stacked with a hole between them. Three ml of tap water was poured into the lower petri plate, and leaves were placed with their blades in the upper petri plate and their petioles poking through the hole into the lower plate and thereby immersed in the water. Filter paper discs having chasmothecia were inverted and attached to the inside of the upper petri dish lid using silicone grease.

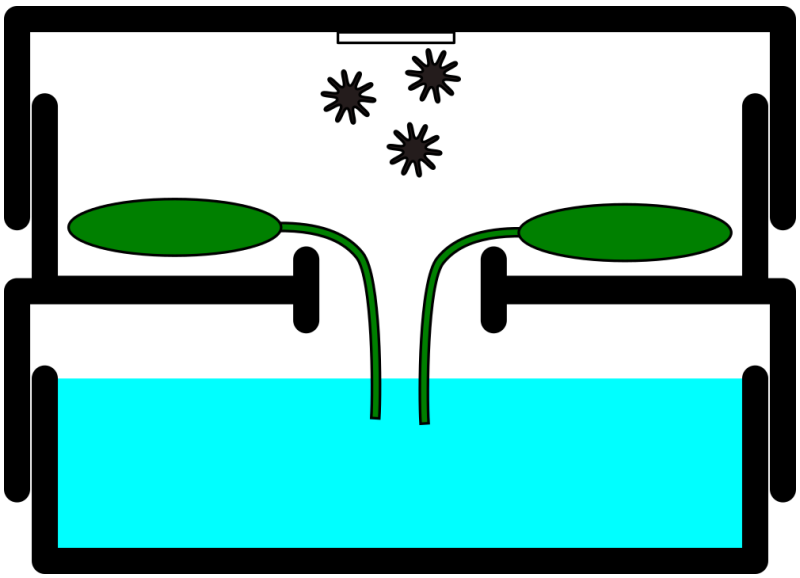


Figure 3: Detached leaf assay with double stacked Petri plate, figure generated using Inkscape version 0.92 (www.Inkscape.org)

Monitoring temperature and relative humidity

Temperature and relative humidity in nurseries were continuously monitored from May to November, with a pair of HOBO pro Series RH/Temp data logger (Onset Computer Corp., Bourne, MA) in the years 2013-2015. Data loggers were placed in a solar radiation shield to protect the external sensors from sunlight and rain and attached

to a pole and placed in the field, one towards north side and other towards the south side of the field each one was kept 50 meters apart. The data loggers monitored the temperature and relative humidity hourly for the duration of each season. Conidia concentration data was plotted against disease severity, temperature and relative humidity to investigate whether there is a correlation between conidia concentration and measured meteorological variables. Wind speed data for the years 2013, 2014 and 2015 starting from the beginning of May to the end of November were obtained from the WSU Agricultural Weather Network (http://weather.wsu.edu) station located at Quincy, WA. Pearson correlation analysis was performed on DNA concentration and weather data using SAS version 9.

Monitoring airborne inoculum

A pair of rotary impaction (Rotorod) air sampling units (Sampling Technologies Inc., Minnetonka, MN) was placed on the leeward sides of cherry during the production seasons of 2013, 2014 and 2015. Both samplers were operated continuously from early May to early September in 2013, and early May to the middle of November during 2014 and 2015. The Rotorod samplers were equipped with two steel rods (4 cm x 1.2 mm) lightly coated with high-vacuum silicone grease (Dow Corning high vacuum grease, Dow Corning Corporation, Auburn, MI) and fastened about 1.5 m above the ground on an aluminum pipe. The two rods on each sampler were collected weekly and combined together for extracting pathogen DNA using the Power soil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, 92010).

Polymerase chain reaction using species-specific primers

PCR was carried out on all DNA samples; DNA that was extracted from the rods of rotary spore traps. The extracted DNA (1µl) was added to a 25µl final volume of PCR cocktail 1X concentration of PCR standard buffer (New England Biolabs, Ipswich, MA), 200µM dNTPs per microliter (New England Biolabs), 400nM gene specific primer and 1.5 U of Taq polymerase. Two different primer sets that were s specific to *P. prunicola* were tested. One primer set was CPM-F (5'-CGT GTG CCG TGT GCA GGT G-3') and CPM-R (5'-CTG GTC ATC GGT TCT CGC TTG T-3') designed by (Liu, Q., 2014 unpublished) were found to amplify a single product of 154 bp specific to *P. prunicola* IGS region. Another primer set was designed by Calabro,J., (2012 unpublished) also specific for *P. prunicola* ITS region was used (Pclan-ITS-89f CTCCACCCGTGTGAACTGA and the reverse primer Pclan-ITS-506r GAGGTCATCCAAAATATATGTGT).

All PCR reactions were conducted using a Biometra T Gradient thermo cycler (Whatman Biometra GmbH, Gottingen, Germany) was used for PCR amplifications. The reactions were cycled between 94°C (initial denaturation) for four minutes, 94°C (DNA denaturation for 45 seconds) 54°C (oligonucleotide primer annealing for 45 sec), and 72°C (Taq polymerase extension for 1 min), and final extension at 72°C for 10 min. DNA denaturation, annealing and extension were repeated for 35 cycles. A negative control without template DNA also was included in each set of PCR reactions.

Quantitative Real time PCR (q RT PCR)

qRT PCR was carried out on all the positive samples (that had shown amplicon with regular PCR). Assay standards were prepared from *P. prunicola* spore/ conidial DNA extractions; five, ten -fold serial dilutions were prepared with DNA concentration ranging from 1.5 to 15,000 pg. Reactions were run in triplicate for both DNA standard and the field samples; DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). qPCR assays were performed on a LightCycler480 system (Roche, Indianapolis, IN) using 96 well plates in a total reaction volume of 10 µl, which consisted of 5 µl of Maxima SYBR green 2X super mix (Thermo Fisher Scientific Inc., Wilmington, DE, USA), 400 nM of each primer (for IGS region) and 1 µl of template DNA. Reaction conditions were 94°C for 10 min, 40 cycles of 15 sec at 94°C, 20 sec at 58°C and 72°C for 30 seconds. For melting curve analysis the cycling conditions include 94°C for 1 sec, 58°C for 1 sec and 60°C for 20 sec. Reactions were conducted in triplicate for all DNA samples including five standard DNA samples and 3 negative control samples. Results were recorded only if no signal was detected from negative control. A lower Cp value (the number of cycles where fluorescence becomes greater than background and measurable) for the qPCR amplification indicated more pathogen DNA (assumed to be spores) collected in an air sample.

Scatter plots were constructed using SAS version 9.2 by taking DNA concentration on Y- axis and day of the year on X- axis (figures 4.1, 4.3 for the year 2013; figures 4.5, 4.7 for the year 2014, figures 4.9, 4.11 for the year 2015). A 3-D graph was generated to represent the spore concentration in the nursery in relation to

temperature and relative humidity using SAS version 9.2 (Figures 4.2 and 4.4 for the year 2013, figures 4.6 and 4.8 for the year 2014 and figures 4.10 and 4.12 for the year 2015).

Results

Cherry seedlings and tree inspection for overwintering chasmothecia

Numerous chasmothecia were observed adhering to woody seedling tissue (2 year old). In 2015 and 2016 plants obtained from storage contained viable chasmothecia. The number and viability of the chasmothecia was high in March compared to April (Table 1). Cherry seedlings planted in growth chamber at various temperatures and high relative humidity did not develop infected (i.e. "flag") shoots during 2015 and 2016; indicating that the fungus is not overwintering in the form of mycelium. These seedlings did not show powdery mildew signs despite of the seedlings carrying viable chasmothecia on the stem surface.

Stored, infested cherry trees (4 year old) had numerous chasmothecia adhering to bark. In the year 2013 the chasmothecia were not viable. During the years 2014-2016; the viability was highest (6-7%) during January. Viability gradually decreased monthly and by June the viability ranged from 0.2-0% (Table 2). Even though dormant cherry trees had numerous viable chasmothecia, they did not develop powdery mildew signs after leaf emergence.

Significance of chasmothecia on cherry leaf debris

The viability of chasmothecia on the cherry leaf debris was high in January during the years 2013 to 2016. The viability decreased to 0.3% by the month of May during all years of the study (Table 3). the leaves carried viable ascospores according to the

microscopic assessment methods but did not produce primary mildew colonies during inoculation experiments.

Effect of temperature and relative humidity on the onset and development of powdery mildew epidemics in the nursery

Powdery mildew signs in cherry nurseries were prevalent when the weekly average temperatures were 21^{o}C (70^{o}F) and decreased when the average temperatures were at or above 28 ^{o}C (83^{o}F). Disease severity and incidence was high when the weekly average temperatures were at 23 ^{o}C -23.8 ^{o}C (74 -75^{o}F), and the average RH was above 55 %. There was a negative correlation between temperature and spore concentration during the 3 the years of study and a positive correlation between relative humidity and spore concentration during the years 2014 and 2015 (Tables 5, 6 and 7). Spore concentration and the wind speed data were correlated, and no correlation was observed.

Molecular detection of airborne spores

DNA was extracted from spores from the middle of May in the years of experiment 2013- 2015. A 150– bp long amplicon was amplified using the IGS primers using regular PCR and *Podosphaera prunucola* specific primers. DNA amplification using cherry powdery mildew specific ITS primers resulted in a 450 bp long amplicon on the 1.8% agarose gel, further confirming the presence of *P.prunicola* spores in the air of the nursery. In 2013, the highest spore concentration occurred in the 3rd week of June (June 19-26). Powdery mildew signs were also apparent during the same time (June 20). Disease severity and incidence was low (5 %) on the untreated control plots. During 2014 powdery mildew symptoms and signs were first observed on July10th; airborne inoculum was detected beginning in mid -May; the inoculum concentration was low in the year

2014 compared to the years 2013 and 2015. In 2015, the highest spore concentrations were observed in the third week of May and powdery mildew signs were observed on June 6th. In 2013 the powdery mildew epidemic started in the month of June and the incidence increased during July. The disease progress curve showed exponential growth in the months July and August and reached a plateau during the final week of August and September (Figure: 2). In the year 2014 disease incidence and severity were low in the month of July and exponential growth was observed during the month of August and it reached plateau in September. In 2015 powdery mildew epidemic started in late June and the incidence gradually increased by mid- July, disease progress curve showed exponential growth in late July and August and reached a plateau during the final week of August and September. The PCR amplicons were 100% identical to the IGS region of *P. prunicola.* There was a positive correlation between spore concentration and disease severity during the years 2013 and 2015 (Tables 5 and 7)

Discussion

The present study was conducted to determine the source of primary inoculum in sweet cherry nurseries. Ascospores are thought to constitute primary inoculum in orchards (Grove, G.G and Boal, R.J 1990). Even though nursery-grown cherry seedlings showed numerous chasmothecia with viable ascospores on bark, powdery mildew signs were not observed in controlled-environment and field studies. Epidemics were evident in nearby orchards before the appearance of symptoms and signs in the nursery. Disease scouting of powdery mildew epidemic in a cherry nursery at a different location (Moses lake WA) also showed that the epidemic in the nursery was 1 month delayed compared to the nearby orchards. Previous work using volumetric spore traps in cherry orchards

revealed the trapping of ascospores until day 117 (First week of May) and the trapping of conidia from mid May (Grove G.G 1998). Sweet cherry orchards located within 5-16 km radius of the cherry nurseries and during the years 2012 to 2016, powdery mildew epidemic in the nursery started 3- 4 weeks after the epidemic initiation in the near by orchards of Quincy. It could be assumed that the air currents carry the *P. prunicola* spores from the nearby orchards to the nursery. The rotary impaction spore traps that were set up in the nursery detected the air borne inoculum and the epidemic was initiated in the nursery after 1 month.

Aerial dispersal is one of the inoculum distributions mechanisms used by the plant pathogens which is not only limited to same field but to other fields and even to a different continent (Pady et al 1955, Gregory, P.H 1973, Brown et al 2002). Studies have shown that powdery mildew conidia can be dispersed over long distances and can cause infections in several crops (Hermansen et al 1978, Schnathorst, (1959c). Powdery mildew signs were seen on the abaxial side of the cherry leaves in the nursery. Spores are deposited on the lower side of the leaf due to turbulent spore deposition (Aylor, D.E., 1974). In the greenhouse *P.prunicola* colonies were observed on adaxial side, whereas in the nursery the colonies were predominantly present on the abaxial side (lower side) of the leaf. In the greenhouse the spores can be splash dispersed during irrigation event and can be deposited on the upper side of the leaf. In nursery rain events are not so common during the powdery mildew epidemic and the spores might be deposited on the lower side of the leaf due to turbulent spore deposition. It could be assumed that viable conidia from the nearby orchards might be carried by air currents to the nearby nurseries and the spores are deposited on the lower side of the leaf.

The chasmothecia present on the cherry leaf debris showed low ascospore viability rates and did not result powdery mildew infections when used as inoculum sources on both attached and detached cherry leaves. The cherry nursery floors were devoid of cherry leaf debris; also the nurseries are rotated from one nursery type to a different nursery type each year. The cherry trees in the nursery are densely grown, the shady canopy and furrow irrigation, actively growing shoots having young susceptible leaves provide ambient conditions for the powdery mildew epidemic. Cherry seedlings with viable chasmothecia on the stems did not develop flag shoots during the years 2015 and 2016 indicating that the seedlings were not carrying any dormant mycelium on them, the seedling did not develop powdery mildew signs despite of providing favorable conditions for the powdery mildew in the growth chamber. Our data indicate that chasmothecia are insignificant in the epidemiology of powdery mildew in Washington cherry nurseries. In the similar manner trees that were harvested from the nursery, despite of having numerous viable chasmothecia on them did not develop powdery mildew signs. It could be assumed that there is a low possibility of fungicide resistance (if exists) spread from nursery to the to the new areas where the nursery trees are transported and propagated as orchards.

Powdery mildews require an optimum temperature of 21^{o}C for conidial germination and for early colony development (Butt, D. J 1978, Celio et al 1997). In this study also it was confirmed that the optimum temperature required for the initiation of powdery mildew epidemics in the cherry nursery was 21^{o}C. Powdery mildew development in wheat decline rapidly with temperatures above 25^{o}C (Jones et al 1983, Wiese, M.V 1987) and in this study also there was negative correlation in the spore

concentration with increasing temperature at or above 28^{o}C. Temperatures of 28^{o}C or above found to be detrimental to the pathogen in case of other powdery mildews too (Quinn et al 1981, Peetz 2009). The DNA concentration in the cherry nursery was positively correlated with relative humidity as; the spore concentration was high when the relative humidity was above 50%.

No correlation was observed between wind speed data and spore concentration possibly due to the fact that the sampling media (rods) in the rotary impaction spore traps were replaced weekly. Once spores/ air borne particles adhere to the adhesive surface, there will not be any extra adhesion surfaces for the new spores/ particles. Reducing the sampling period (to once in every 24 hours) would have provided valuable information on spore release data but the location of the nursery was a limiting factor: as the nurseries are located 155 km away from the research station. Volumetric spore samplers would have provided valuable information on wind speed data and temporal spore concentration. Rotary impaction spore traps are useful for diagnostic purposes but they have limitations such as they do not provide information on what time / period of the day the spore concentration is high or low.

Cherry trees harvested from the nursery and stored over winter had numerous chasmothecia with viable ascospores on woody stems. When planted either in the greenhouse or in the field powdery mildew symptom and signs developed after the bud break and no flag shoots were observed. These findings are similar to those of Grove et al, 1991. Our results indicated that there is a low probability of nursery stock distributing overwintering inoculum to the new growing areas via nursery shipments.

Our present work in sweet cherry nurseries indicate that the air borne spores from external sources are responsible for the initiation of powdery mildew epidemics in sweet cherry nurseries. Disease scouting in the nursery had revealed that epidemic in nursery developed three to four weeks after the epidemic initiation in the cherry orchards. From cherry seedling washings data, disease scouting data and rotary spore impaction data, it was concluded that air borne spores are the main source of initial inoculum in the sweet cherry nurseries.

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Table 1: occurrence of *P. prunicola* chasmothecia on the cherry seedlings in the month of March and April during the years 2015 and 2016. Number and percent viable of ascospores recorded during the years 2015 and 2016

	2015		2016	
	Average Number of chasmotheica	Percent viable	Average Number of chasmotheica	Percent viable
March	73	4.3	31.6	5.6
April	15.5	2	24.67	5.63

Table 2: occurrence of *P. prunicola* chasmothecia on the cherry tree stems from storage, number and percent viable of ascospores recorded during the years 2013 to 2016

	2013		2014		2015		2016	
	Average # of chasmotheica	% viable	Average Number of chasmotheica	Percent viable	Average Number of chasmotheica	% viable	Average Number of chasmotheica	Percent viable
January	59	0	92.1	6.3	174.8	6.72	141	7.2
February	44.7	0	39	1	78	1.5	284.125	5.375
March	22.8	0	40.6	1.8	160	1.3	212.7	0.3
April	17	0	58	0.7	111.1	0.1	177.8	3.6
May	4.5	0	108.3	0.2	25	0	224.5	0.2

Table 3: occurrence of *P. prunicola* chasmothecia on the cherry leaves that were kept in a cage from previous season, percent viability of ascospores recorded during the years 2013 to 2016. The viability is high in January and goes down by the month of June.

	Year			
	Average number of Chasmothecia from caged leaves			
	2013	2014	2015	2016
January	9	13	16	27.2
February	7	10.3	17.3	23.8
March	6	8.3	4.7	6.4
April	4.3	6.7	2.7	3.2
May	4	5.3	0.3	0.7
June	2	2.3		

Table 4: represents powdery mildew epidemic initiation in the nursery and in the nearby orchards in Quincy, WA. As seen in the table powdery mildew epidemic in the orchards starts earlier than in the nurseri

Powdery mildew scouting	Year				
	2012	2013	2014	2015	2016
Nursery	18-Jun	27-Jun	10-Jul	6-Jun	1-Jul
Orchard	May 15	May 18	29-May	17-May	8-Jun

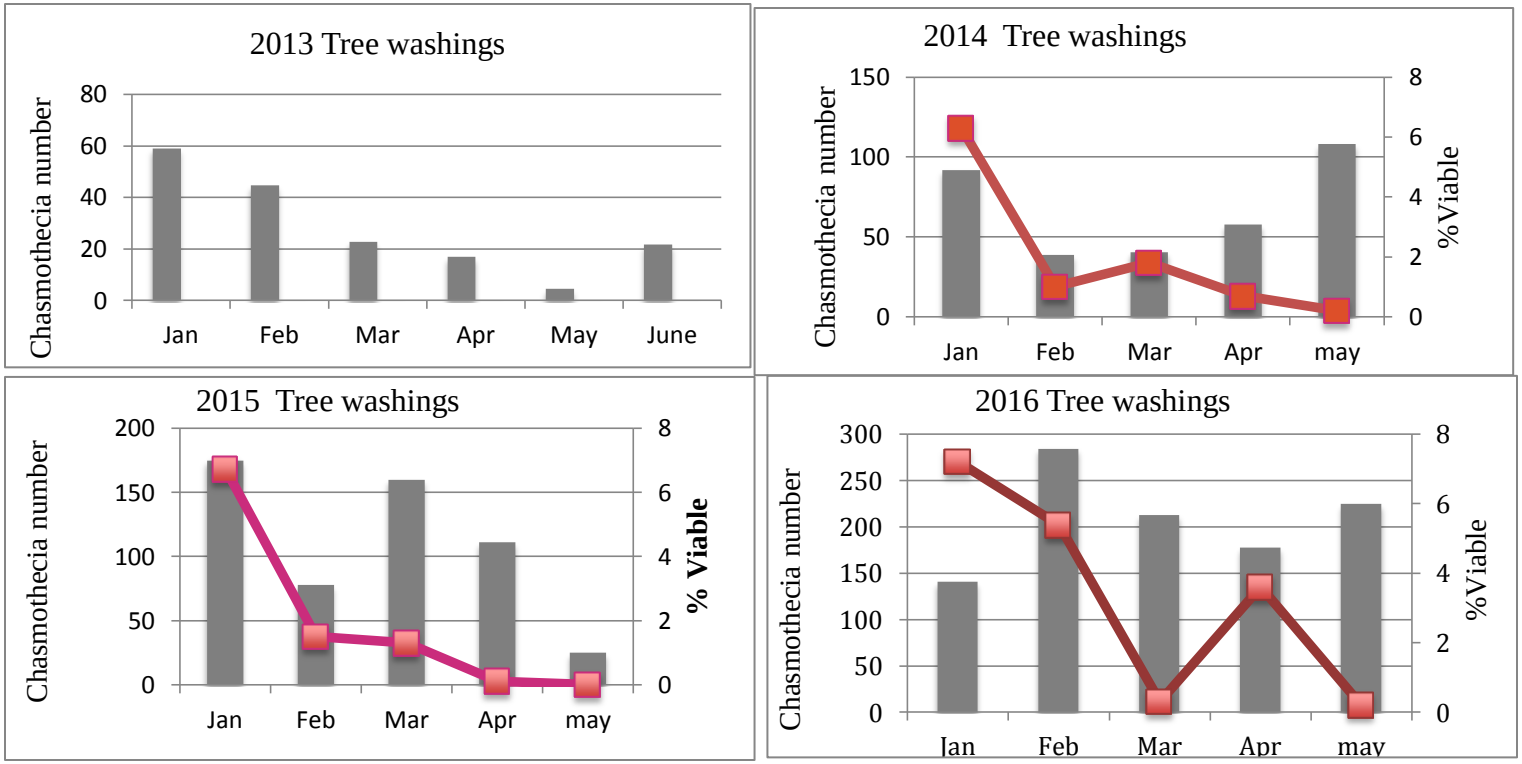
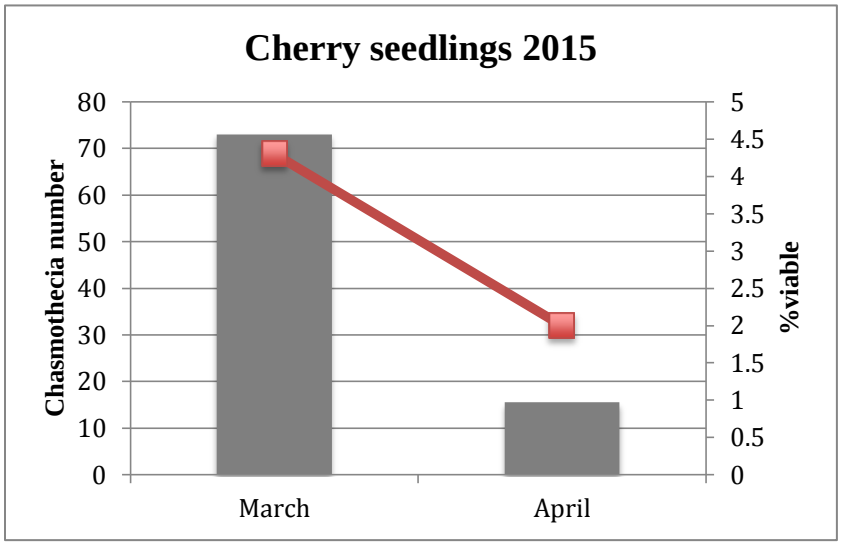


Figure 4: Mean chasmothecia number isolated from the cherry stems each month beginning from the month January to June during the years 2013 to 2016. On the secondary axes represent the percent viable ascospores from the chasmothecia from the month January to June during the years 2013 to 2016. In 2013 none of the chasmothecia were viable. During the years 2014 to 2015, the viability is high (6-7%) in the beginning of January and viability percent decreases each month; by the month of May the viability reaches to 0%.



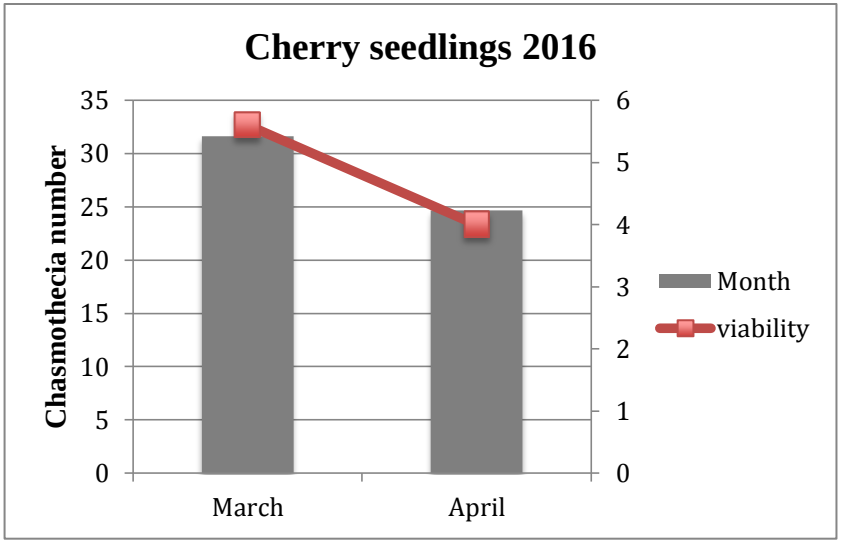


Figure 5: Mean chasmothecia number isolated from the cherry seedlings stems during the months March and April during the years 2015 and 2016. The secondary axes represent the percent viable ascospores from the chasmothecia from the month March to April during the years 2015 and 2016. Percent viable chasmothecia decreases during course of time.

Table 5: Pearson correlation analysis for concentration of the conidia, disease severity, temperature and relative humidity using SAS version 9 for the year 2013. Disease severity and the spore concentration are significantly correlated. P≤.05 and correlation coefficient is 64%

Pearson Correlation Coefficients **Number of Observations**					
	Day	**Severity**	**Conc**	**RH**	**Temp**
Day	1 16				
Severity	0.73713 0.0096 11	1 11			
Concentration	0.41679 0.1222 15	0.64116 0.0335 11	1 15		
RH	0.36229 0.1845 15	0.12591 0.7122 11	0.28457 0.304 15	1 15	
Temperature	0.46971 0.0664 16	0.43374 0.1826 11	0.14825 0.598 15	-0.3649 0.1811 15	1 16

Table 6: Pearson correlation coefficient among conidia concentration, disease severity and environmental factors for the year 2014. Spore concentration and relative humidity are significantly correlated ($p \leq 0.05$) and correlation coefficient is 50.2%; temperature and relative humidity were negatively correlated, $P \leq 0.05$ and correlation coefficient is-54.6%

Pearson Correlation Coefficients Number of Observations					
	Day	**Concentration**	**Severity**	**Temp**	**RH**
Day	1 23				
Concentration	0.49642 0.016 23	1 23			
Severity	0.64855 0.0049 17	0.77542 0.0003 17	1 17		
Temp	-0.2763 0.2132 22	-0.2016 0.3684 22	-0.2965 0.2479 17	1 22	
RH	0.73473 <.0001 22	0.50236 0.0172 22	0.23934 0.3549 17	-0.5469 0.0084 22	1 22

Table 7: Pearson correlation coefficient among conidia concentration, disease severity and environmental factors for the year 2015. Spore concentration and relative humidity are significantly correlated ($p \leq 0.05$) and correlation coefficient is 50.2%; temperature and relative humidity were negatively correlated, $P \leq 0.05$ and correlation coefficient is-

Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations					
	Day	**Concentration**	**Temperature**	**RH**	**Severity**
Day	1 19				
Concentration	0.16673 0.4951 19	1 19			
Temperature	-0.8156 <.0001 19	-0.1892 0.4379 19	1 19		
RH	0.70666 0.0007 19	0.29843 0.2146 19	-0.7218 0.0005 19	1 19	
Severity	0.85935 <.0001 15	0.49098 0.0631 15	-0.7469 0.0014 15	0.7919 0.0004 15	1 15

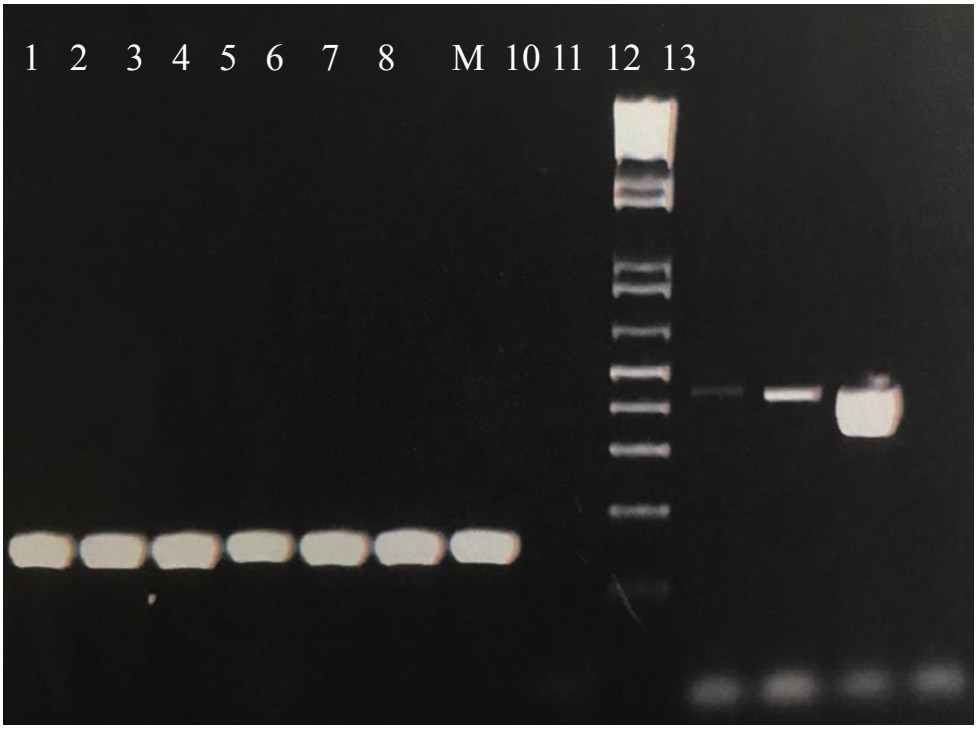


Figure 6: Amplification of Intergenic specific region of *P. prunicola;* amplicons resolved on 1.5 % agarose gel. Lanes 1 to 7 were amplified with primer pair that is specific for *P. prunicola* IGS region. Lane 8 is water control and lane 9 is 1 –KB- plus DNA marker (Invitrogen Corporation, Carlsbad, CA, USA) for estimating the size of amplicon. Lanes 10 to 12 were amplified with primer pair primer pair that is specific for *P. prunicola* ITS region designed by Calabro, J., 2012; lane 13 is water control.

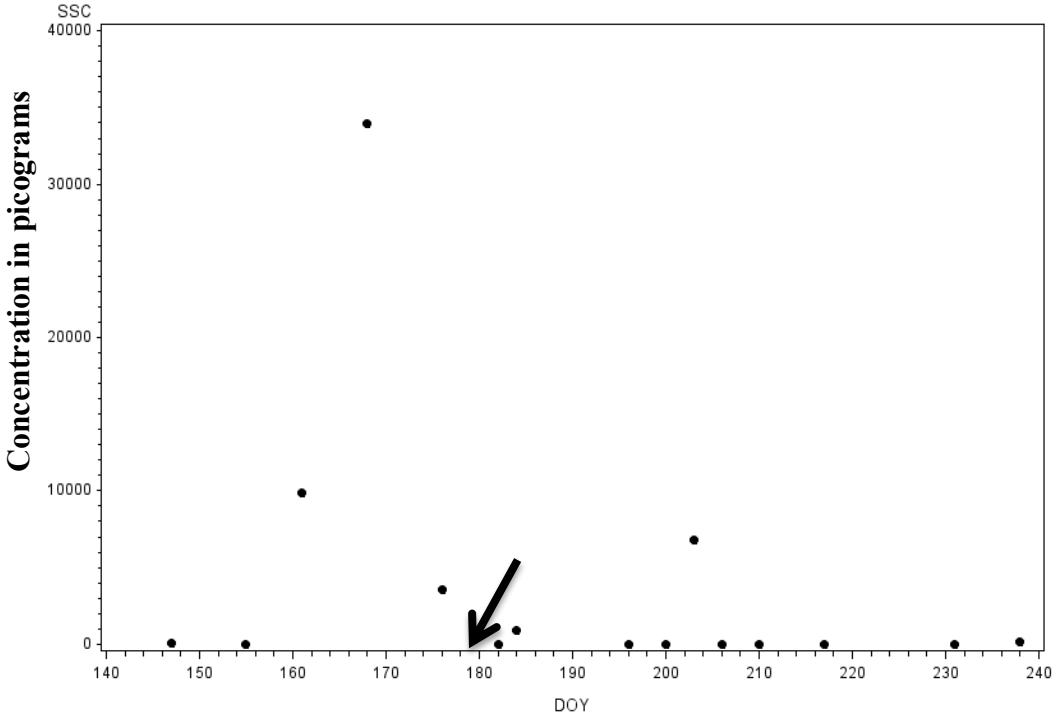


Figure 7: Scatter plot diagram showing concentration of DNA in picograms. The concentration of the DNA was quantified using qRT-PCR. DNA was extracted from the rotary impaction spore traps placed on the north side of the cherry nursery in the year 2013. The arrow represents the observance of powdery mildew signs in the sweet cherry nursery. X axis represents the day of the year. Powdery mildew spores were detected in the nursery even before the appearance of powdery mildew signs. This diagram was generated using SAS program version 9.2.

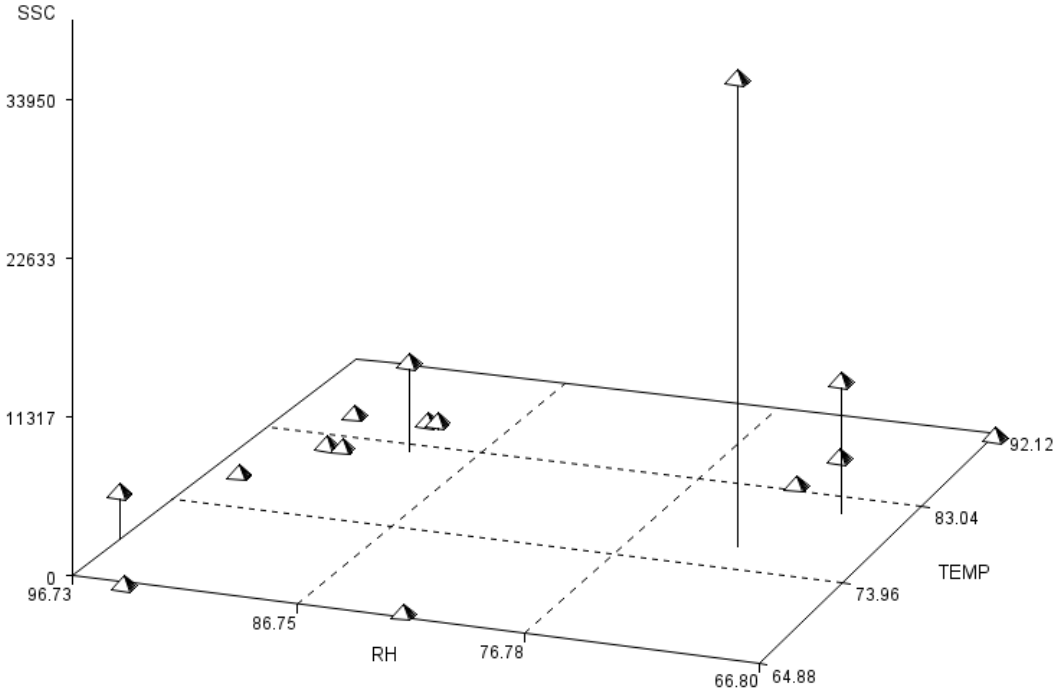


Figure 8: 3-D diagram generated using SAS version 9.2. This figure depicts the concentration of cherry powdery mildew spores from the north side of the cherry nursery during the year 2013 in relation to temperature and relative humidity. When powdery mildew signs in cherry nursery appeared, the weekly average temperatures were 21^{o}C (70^{o}F) with a relative humidity >75%.

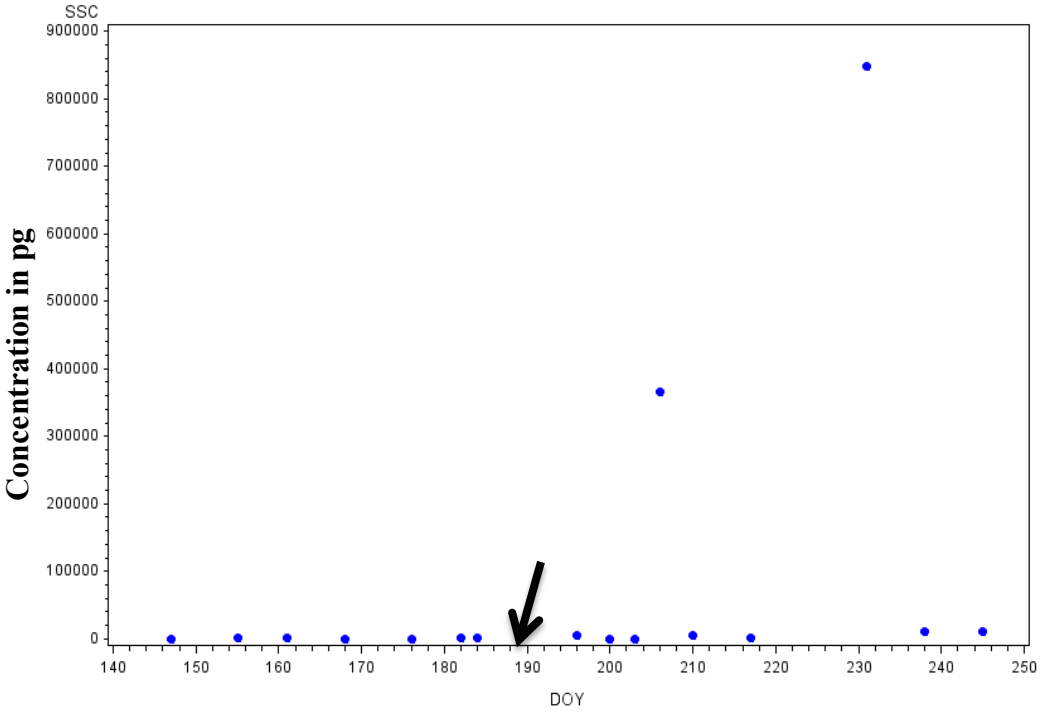


Figure 9: Scatter plot diagram showing concentration of DNA in pg. The concentration of the DNA was quantified using qRT-PCR. DNA was extracted from the rotary impaction spore traps placed on the north side of the cherry nursery in the year 2013. Arrow represents the observance of powdery mildew signs in the sweet cherry nursery. X-axis shows the day of the year, powdery mildew spores were detected in the nursery even before the powdery mildew appearance. This diagram is generated using SAS program version 9.2.

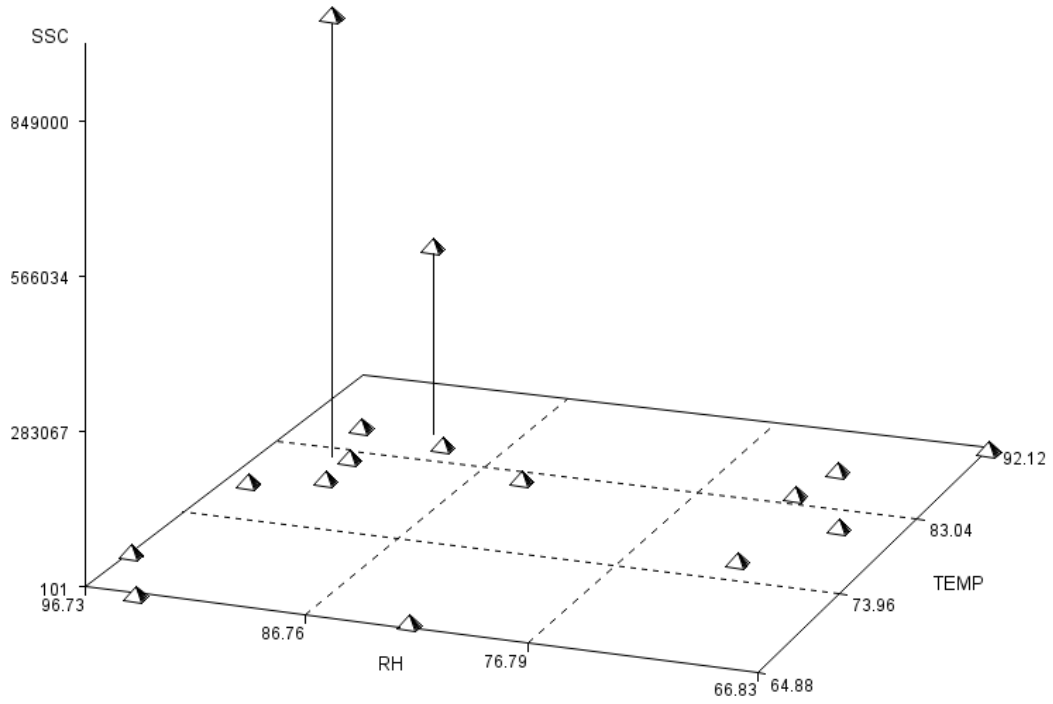


Figure 10: 3 -D diagram generated using SAS version 9.2, this figure depicts the concentration of cherry powdery mildew spores from south side the cherry nursery during the year 2013 in relation to temperature and relative humidity. Powdery mildew signs in cherry nursery appeared when the weekly average temperatures were 21^{o}C (70^{o}F) with high relative humidity above 70%

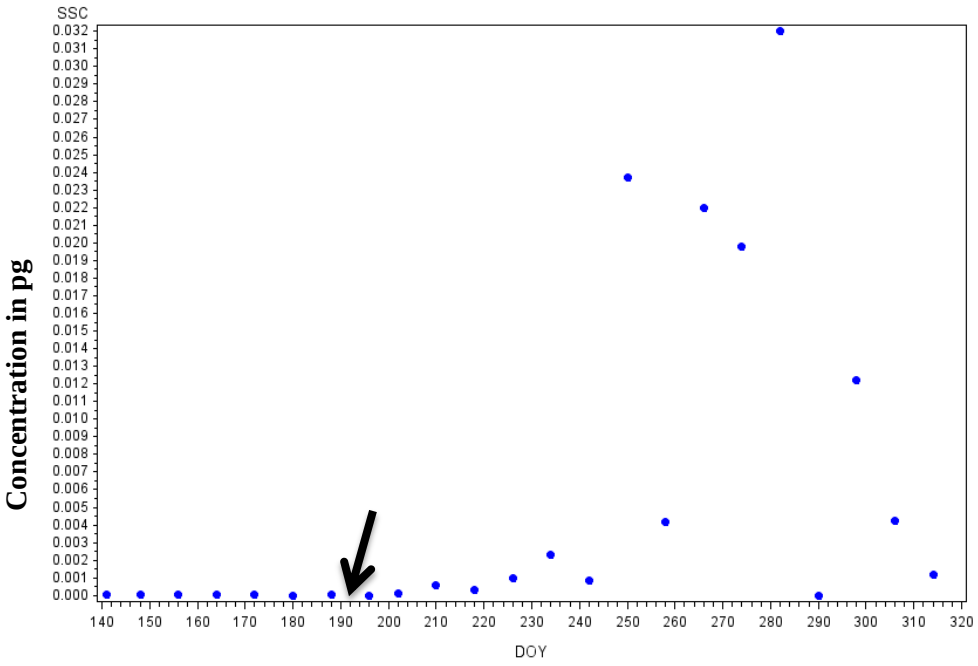


Figure 11: Scatter plot diagram showing concentration of DNA in picograms. The concentration of the DNA was quantified using qRT-PCR. DNA was extracted from the rotary impaction spore traps placed on the north side of the cherry nursery in the year 2014. Arrow represents the observance of powdery mildew signs in the sweet cherry nursery. X axis shows the day of the year, powdery mildew spores were detected in the nursery even before the powdery mildew appearance. This diagram is generated using SAS program version 9.2.

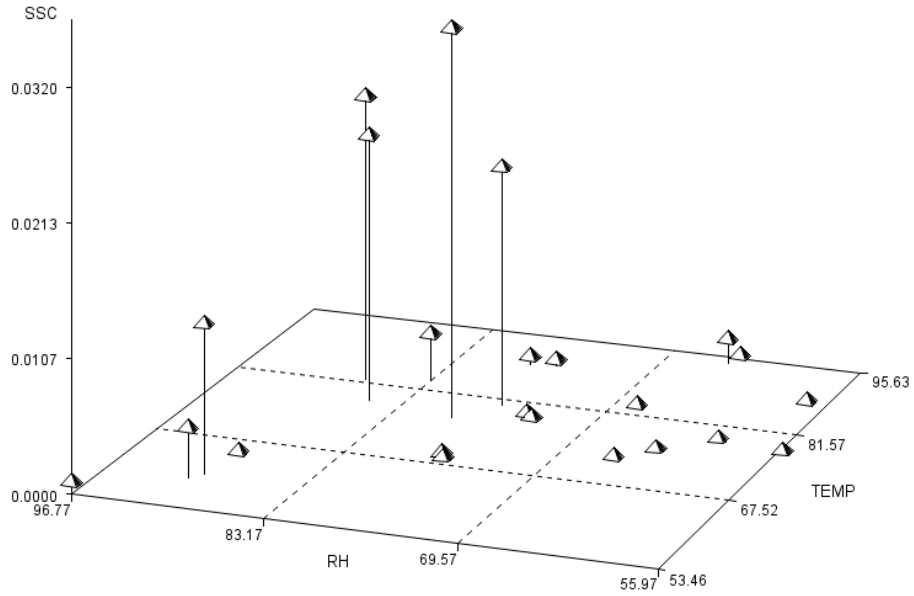


Figure 12: 3 -D diagram generated using SAS version 9.2, this figure depicts the concentration of cherry powdery mildew spores from north side of the cherry nursery during the year 2014 in relation to temperature and relative humidity. Powdery mildew signs in cherry nursery appeared when the weekly average temperatures were 21^{o}C (70^{o}F) and at high relative humidity above 67%.

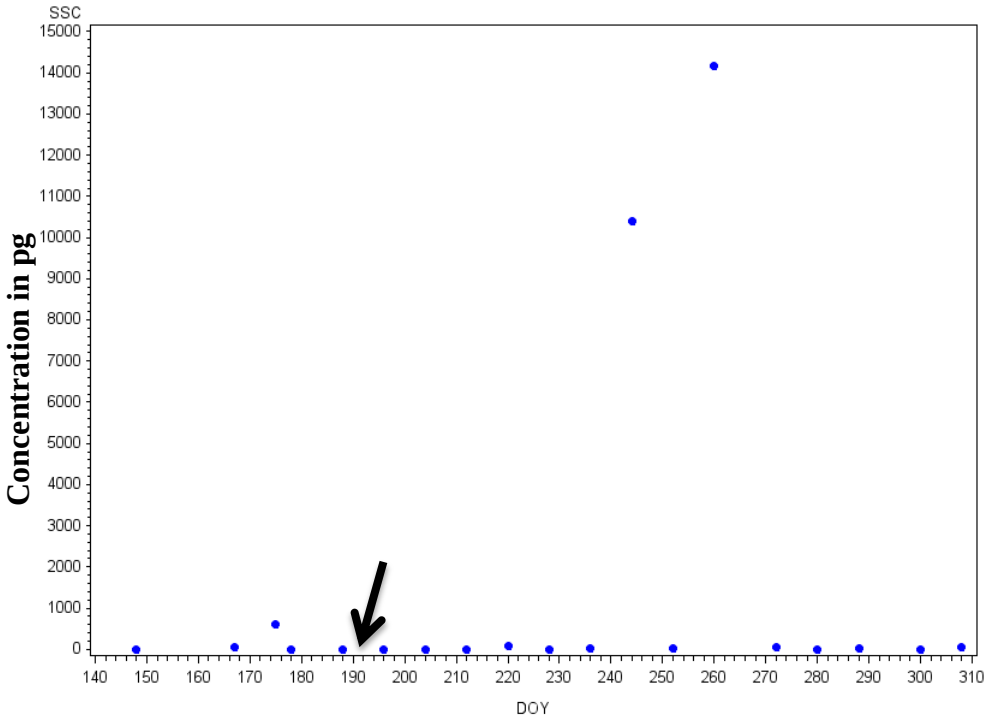


Figure13: Scatter plot diagram showing concentration of DNA in picograms. The concentration of the DNA was quantified using qRT-PCR. DNA was extracted from the rotary impaction spore traps placed on the north side of the cherry nursery in the year 2014. Arrow represents the observance of powdery mildew signs in the sweet cherry nursery. X axis shows the day of the year, powdery mildew spores were detected in the nursery even before the powdery mildew appearance. This diagram is generated using SAS program version 9.2.

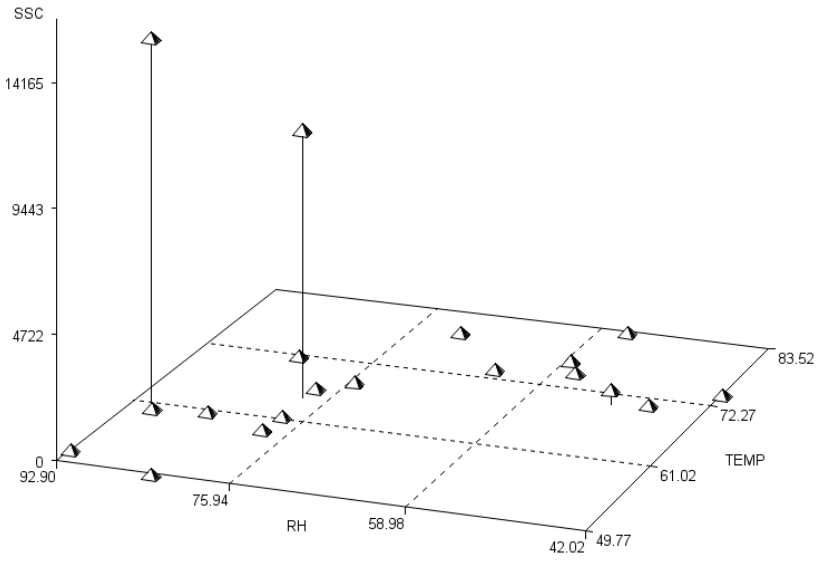


Figure 14: 3 -D diagram generated using SAS version 9.2, this figure depicts the concentration of cherry powdery mildew spores from south side of the cherry nursery during the year 2014 in relation to temperature and relative humidity. Powdery mildew signs in cherry nursery appeared when the weekly average temperatures were 21^{o}C (70^{o}F) and at relative humidity above 61%.

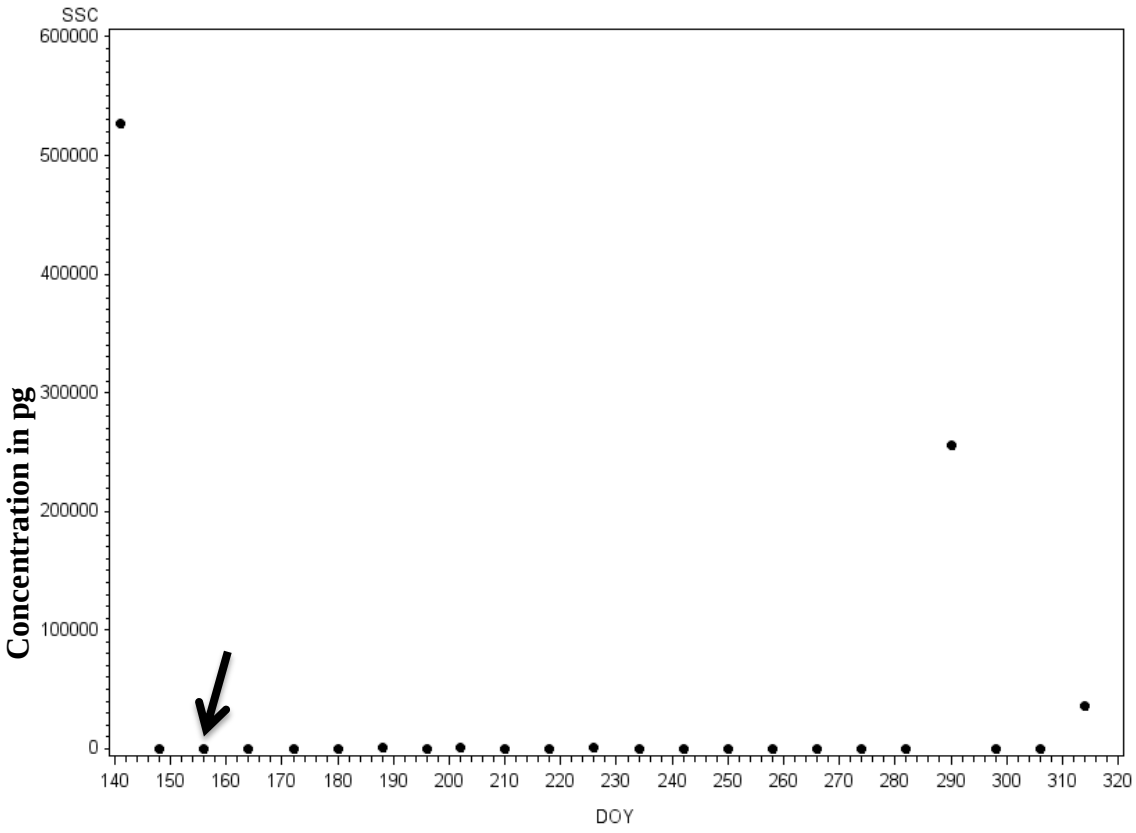


Figure 15: Scatter plot diagram showing concentration of DNA in picograms. The concentration of the DNA was quantified using qRT-PCR. DNA was extracted from the rotary impaction spore traps placed on the north side of the cherry nursery in the year 2015. Arrow represents the observance of powdery mildew signs in the sweet cherry nursery. X-axis shows the day of the year, powdery mildew spores were detected in the nursery even before the powdery mildew appearance. This diagram is generated using SAS program version 9.2

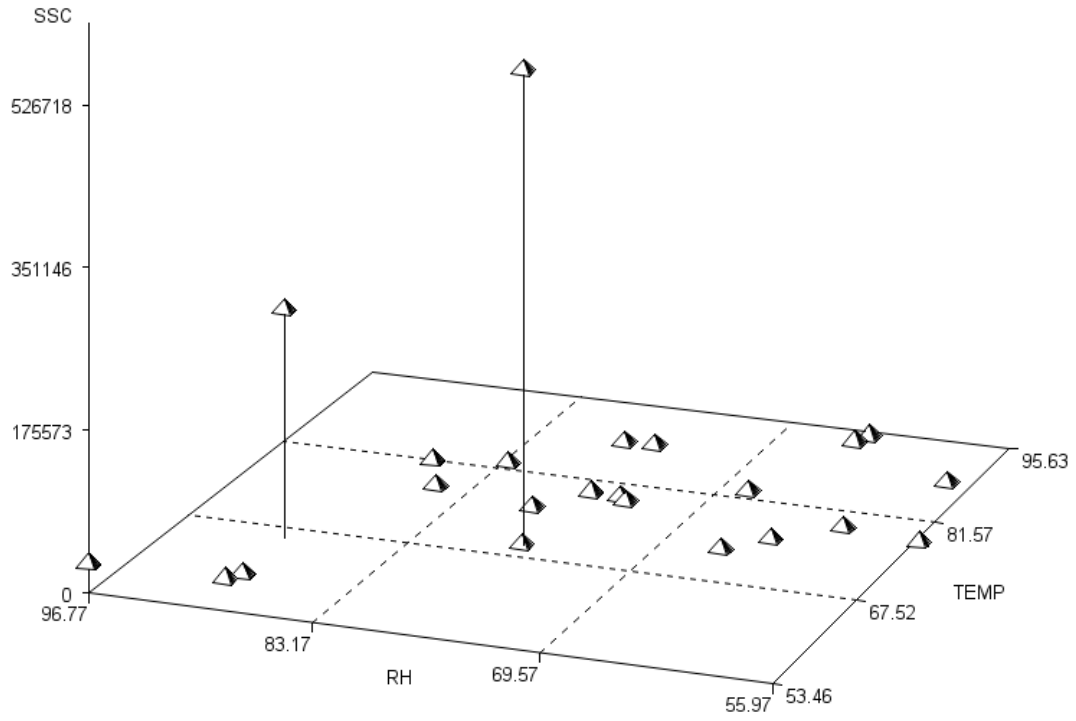


Figure 16: 3 -D diagram generated using SAS version 9.2, this figure depicts the concentration of cherry powdery mildew spores from north side of the cherry nursery during the year 2015 in relation to temperature and relative humidity. Powdery mildew signs in cherry nursery appeared when the weekly average temperatures were 21^{o}C (70^{o}F) and at high relative humidity 68%.

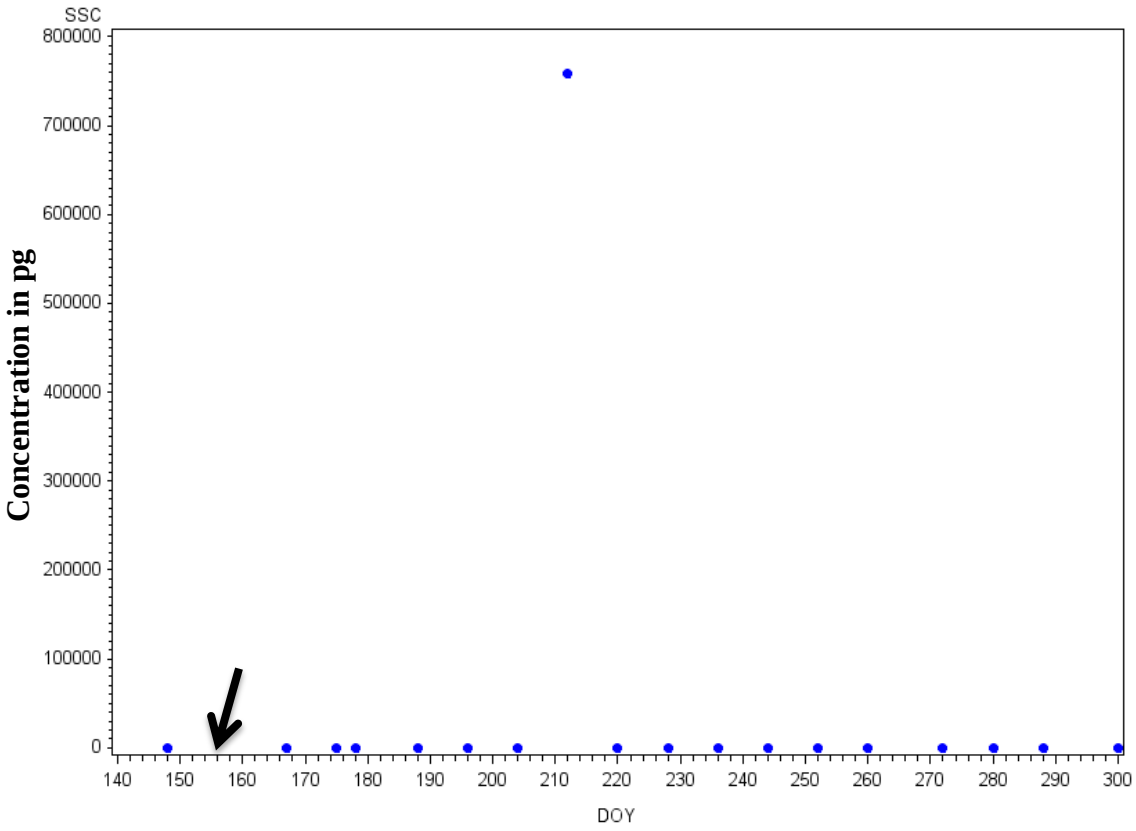


Figure 17: Scatter plot diagram showing concentration of DNA in picograms. The concentration of the DNA was quantified using qRT-PCR. DNA was extracted from the rotary impaction spore traps placed on the south side of the cherry nursery in the year 2015. Arrow represents the observance of powdery mildew signs in the sweet cherry nursery. X axis shows the day of the year, powdery mildew spores were detected in the nursery even before the powdery mildew appearance. This diagram is generated using SAS program version 9.2

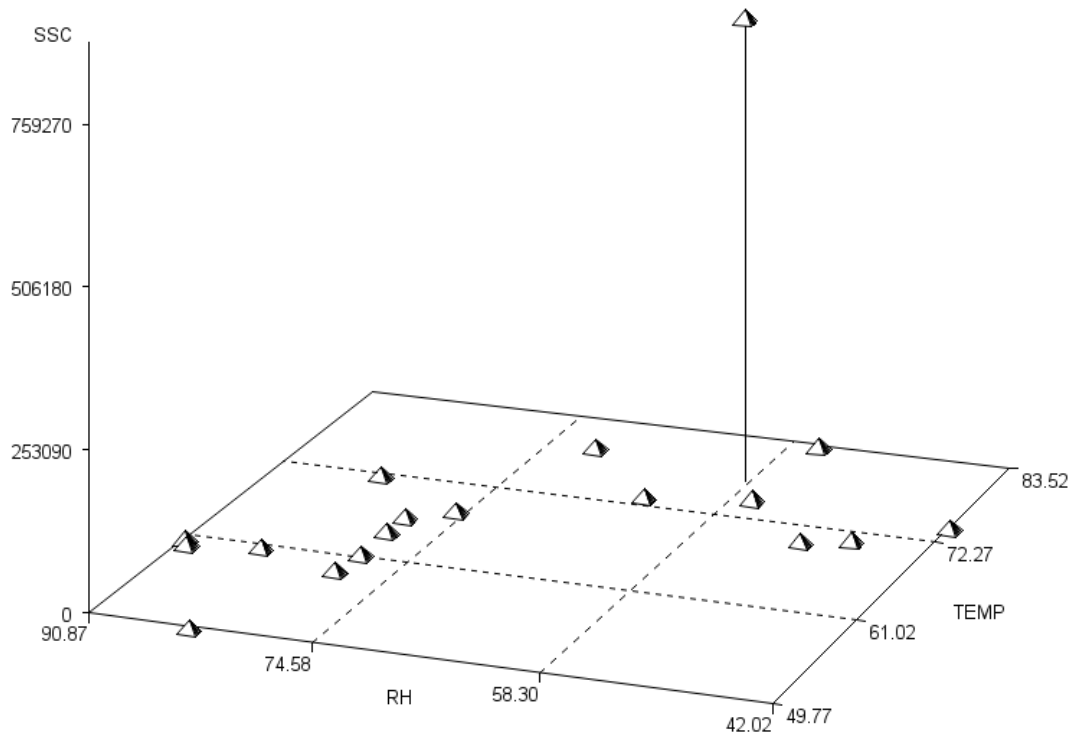


Figure 18: 3-D diagram generated using SAS version 9.2, this figure depicts the concentration of cherry powdery mildew spores from south side the cherry nursery during the year 2015 in relation to temperature and relative humidity. Powdery mildew signs in cherry nursery appeared when the weekly average temperatures were 21^{o}C (70^{o}F) and at a relative humidity above 61%.

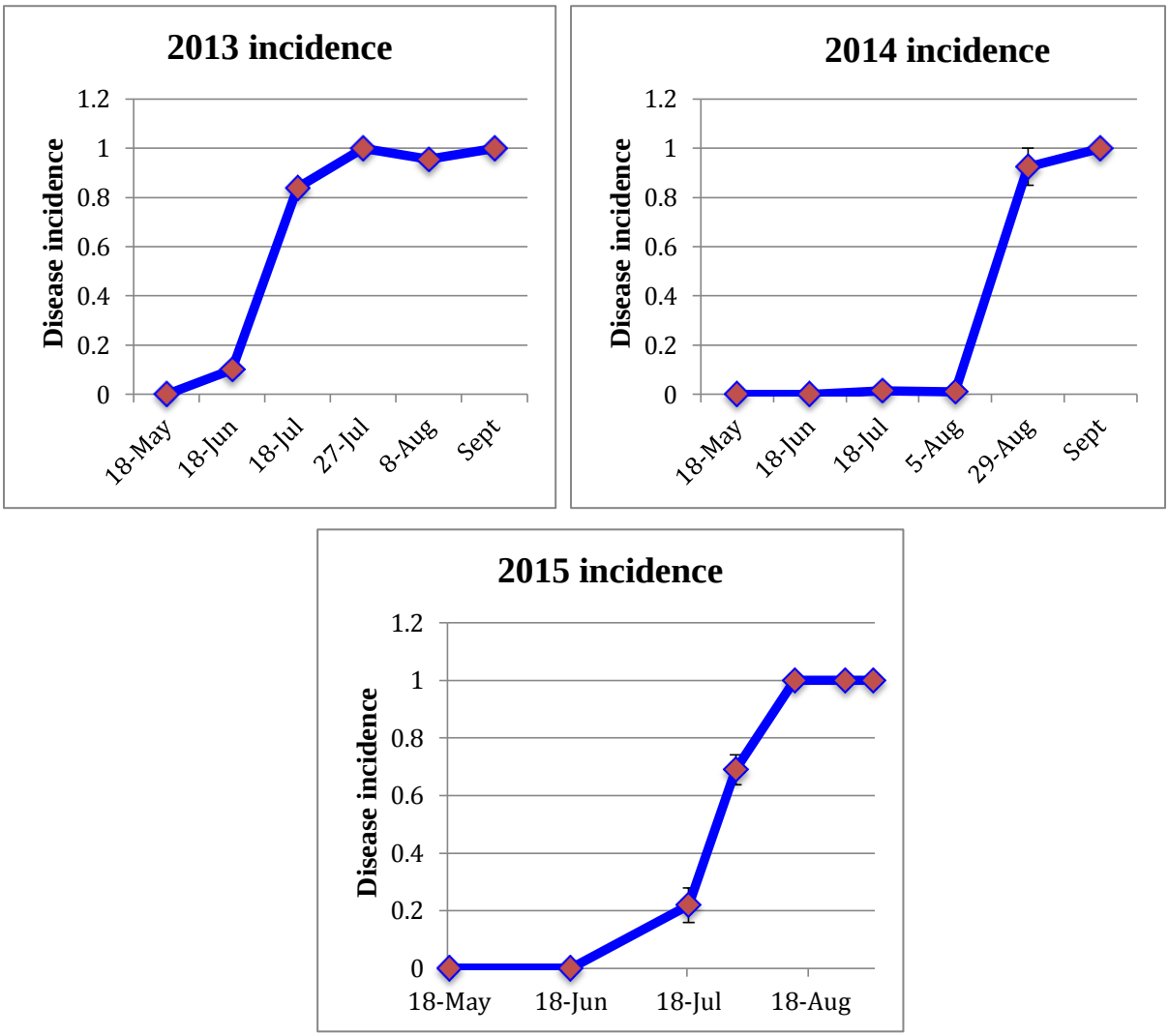


Figure 19: Disease progress curves obtained from disease ratings of cherry powdery mildew during the years 2013 to 2015. Disease ratings were conducted beginning from the month of May until the month of September. Powdery mildew epidemic started in the middle of June in the years 2013 and 2015; in the middle of July for the year 2014.

Exponential rate of disease progression can be observed from middle of July until mid August, there after the disease progress reaches plateau phase.

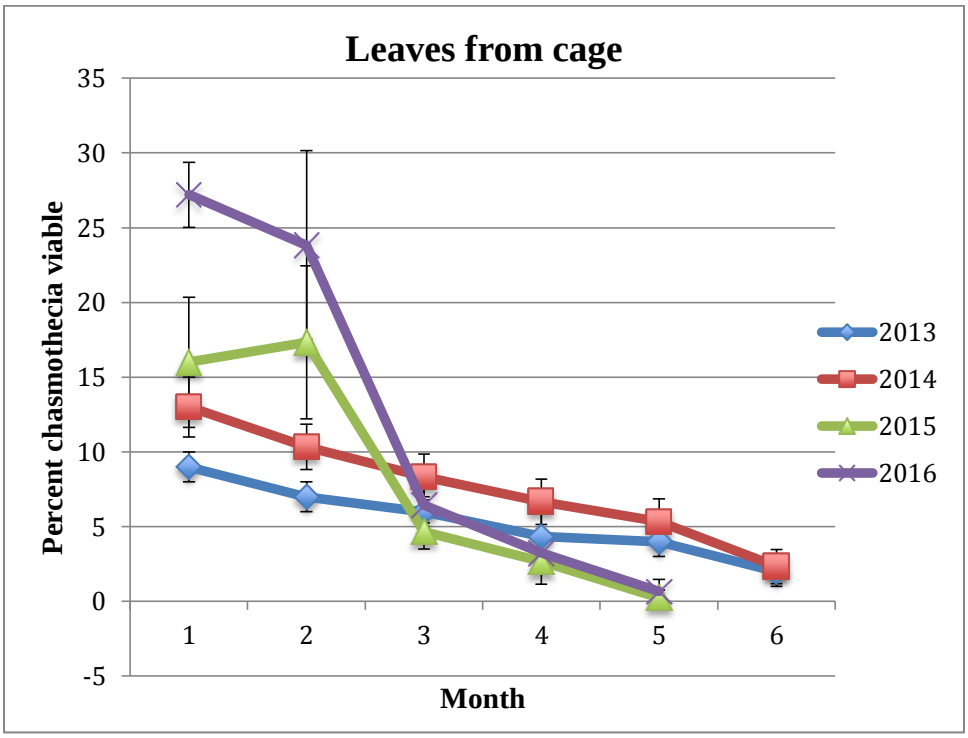


Figure 20: chasmothecia viability reduces from the month January till May (June for the years 2013& 14) during the years 2013 to 2016. Ascospore viability is high at the beginning and goes down each month and most of the chasmothecia are not viable by the end of May.

CHAPTER FOUR

MANAGEMENT OF POWDERY MILDEW IN SWEET CHERRY NURSERIES USING BIORATIONAL PRODUCTS

Abstract

Powdery mildew is the most important foliar disease in sweet cherry nurseries in Eastern Washington. Cherry nurseries are heavily infested with *Podosphaera prunicola* and the disease is managed by spraying the synthetic fungicides on a regular basis. With the emergence of powdery mildew resistance to Quinone outside inhibitor (QoI) and demethylation inhibiting (DMI) fungicides, there is a great need to rely on alternative methods to synthetic fungicides. Recently electrolyzed oxidized (EO) water has proven to be effective against foliar pathogens including powdery mildew. The effectiveness of 2 different bio fungicides, EO water and late season spray oil was investigated. There was a significant decrease in the disease severity and chasmothecia number ($p<0.05$) when EO water was applied weekly in the green house, nursery and orchard. Biweekly application of EO water was effective at low disease pressure but not effective in suppressing powdery mildew and chasmothecia number. Late season application of spray oils has shown a significant decrease in the chasmothecia number on the foliage ($p<0.01$), but the viability was not affected when compared to untreated trees. Bio fungicides (*Bacillus subtilis* and *B. pumilis*), and Actigard were ineffective in controlling the powdery mildew in green house and in field trials. The number of chasmothecia on the stem were significantly lower ($p<0.05$) on the trees that were treated with fungicides (Quinoxyfen,

Fluopyram/ Trifloxystrobin) compared to the untreated trees during both years of the study.

Introduction

Powdery mildew of sweet cherry, which is caused by *Podosphaera prunicola,* is a major disease in the cherry nursery production of Eastern Washington. The conditions that are favorable for powdery mildew epidemic are warm daytime temperatures (21-25°C) and cold nighttime temperatures (13-15°C), closed plant canopy and poor air circulation (Tija, B. 1984, Celio et al 1997). Because of close plant spacing and restricted airflow, cherry nurseries provide favorable conditions for powdery mildew epidemics. Disease management in commercial nurseries is accomplished primarily by the use of intense fungicide programs. Commonly used fungicides are Quinone outside Inhibitors (QoIs), sterol demethylation inhibitors (DMI) and quinoline fungicides. Control of powdery mildew using synthetic fungicides can be expensive due to increased, chemical, fuel and labor costs (Ball and Folwell, 2003). Powdery mildew is generally managed by using the synthetic fungicides and there is possibility that the powdery mildew fungi can develop resistance against the fungicides at the nursery. Fungicide resistance was observed in different powdery mildew such as *Blumeria graminis, P.xanthii* and *E.neacator* (Bletter et al 1998, Brown, J.K.M., 2002). Chemical control is not always effective and there is a possibility of emergence of pathogen populations resistant to some fungicides (Gullino and Wardlow, 1999; LaMondia, J.A., and Douglas, S. M., 1997; Yourman, L. G., and Jeffers, S.N. 1999; Hoffmann et al 2009). In California, USA, isolates of *uncinula necator* showed resistance to triadimefon, myclobutanil and fenarimol (Gubler et al 1996). A similar study conducted in New York, USA, DMI

resistant isolates of *E.necator* showed high ED 50 values to azoxystrobin and mcyclobutanil and it was termed as cross-sensitivity (Wong, F.P., Wilcox, W. F., 2002). Another study on cucurbit powdery mildew isolates (*Podosphaera xanthii*) showed decreased sensitivity to DMI fungicides (McGrath, M.T., Shishkoff, N. 2001). Apart from this, there are other implications of using fungicides regarding environment and potential worker safety (Gullino et al 1999).

Biofungicides are derived from microbial or biochemical products that are obtained from bacteria, plants, animal, or minerals. Studies conducted by Utkhede and Koch (2006) has shown that *Bacillus subtilis* significantly reduced powdery mildew severity in cucumber compared to untreated plants. Recent study with biopesticides had shown that they were effective in preventing foliar diseases on cucurbit seedlings in green houses (Keinath, A.P., 2016).

Paraffinic / mineral oils have been in use in the control of powdery mildew and it was proven effective in reducing the powdery mildew of apple, cherry, cucurbits, grapes and roses (Calpouzos et al1966, Fernandez et al., 2006, Grove et al., 2005, Pasini et al., 1997) Application of stylet oil to cucurbit powdery mildew resulted the death of conidia and mycelium in the center of lesion, after several days of treatment (McGrath, M., Shishkoff, N., 2000)

In recent years Electrolyzed Oxidizing (EO) water emerged as an alternative to fungicides (Mueller et al 2003). Passing a diluted salt solution through the electrodes of an electrolytic cell generates EO water. The electrodes (anode and cathode) are partitioned by a bipolar membrane, one stream of water is alkaline and other stream of

water has unique properties such as low pH ranging from 2.3 (intense)-6.5 (mild), high oxidation reduction potential, improved permeating ability, effective electron scavenger and effective antimicrobial (Hati et al 2012). EO water is used in food industry to sanitize and increase the shelf life of the produce (Vicente, M., Gomez-Lopez 2012). EO water did not cause phytotoxicity in 15 species of bedding plants when applied 2 times (Buck et al 2002). EO water was proven to be effective on apples against post harvest pathogens such as *Pencilium expansum* (Okull et al 2004). EO water has proven to be effective against the powdery mildew in the green house grown Gerbera Daisy (Mueller et al 2003).

Objectives

The objective of the present study is to determine the effectiveness of bio fungicides, paraffinic oil, and EO water in managing the powdery mildew and suppressing chasmothecia numbers.

Materials and Methods

Nursery trial at Quincy, WA

A commercial cherry nursery (Van Well nursery) located 5 km west of Quincy was used for the efficacy of different biological treatment studies from 2012 to 2016. The nursery comprised of 4-year-old cherry trees cv. Bing on Mazzard rootstock.

Cherry nursery trial 2012, 2014 and 2015

Efficacy trials were designed as randomized complete block design with 4 replications, and 10 plants per treatment. In the year 2012, efficacy of EO water was tested against 2 standard fungicides (Quinoxyfen, Fluopyram/ Trifloxystrobin). The

experiment was designed as randomized complete block design, each replication had 4 treatments and 10 plants per treatment. Four treatments included, one control, EO water treatment and 2 standard fungicide treatments. Fungicide rates were calculated on the spray volume of 3,785 L/ ha (1 gallon/ 2.47 acres). Fungicide rate per gallon water is shown in Table 1. Treatments were started before epidemic development (first week of June 2012 to 2015) and were applied biweekly. EO water was obtained from Weber farms - Quincy WA; the water was generated from a machine called AquaFew EO Water Technology. The machine uses an Anode/Cathode membrane. Low pH water is on positive side i.e anode side of membrane was collected for this study. EO water and 2 standard fungicides (chemical name: 5,7-dichloro-4-p-fluorophenoxy quinolone, trade name: Quintec, Dow AgroSciences LLC, Indianapolis, IN, 2. Chemical name: Fluopyram, trifloxystrobin, trade name: Luna sensation Fluopyram/ Trifloxystrobin, Bayer, Research triangle park, NC) were sprayed in a 2 week interval; control trees were sprayed with tap water. Treatments were applied to run off using a 15.14-liter backpack sprayer with a piston pump. A separate backpack sprayer was used for EO water applications; treatments were applied until the end of August.

In the year 2014, each replication had 4 treatments and each treatment had10 plants. The treatments include, 1.Control 2. *Bacillus subtilis*, 3. *Bacillus subtilis* alternated with a Fluopyram/ Trifloxystrobin and 4. A standard fungicide- Fluopyram/ Trifloxystrobin.

In the year 2015, each replication had 6 treatments and each treatment had 10 plants. Six treatments were tested in each replication; the treatments include 1.Actigard 2. EO water 3. Fluopyram/ Trifloxystrobin 4. *Bacillus subtilis* 5. *Bacillus pumilis* 6.

Control. EO water was sprayed in a 1-week interval (this is contrast to 2012) and the remaining treatments were sprayed in 2-week interval (Table 1.1).

Green house trial in 2015

Cherry (cv. Bing) plants 4 yrs old and one foot tall (0.3048 meter long) were planted on April 25th and 26th in the year 2015 in 18.9 liter pots (5 gallon) and were placed on green house bench. The plants were watered regularly, after the bud break the plants were covered with 5 μm of mesh Nitex cloth to prevent infection by conidia of *P. prunicola*. The experiment was arranged in a randomized complete block design, with 4 replications and 5 plants (observations) per replication. Seven treatments were tested in each replication; the treatments include 1. Actigard 2. EO water 3. Onion extract 4. Fluopyram/ Trifloxystrobin 5. *Bacillus subtilis* 6. *Bacillus pumilis* and 7. Control. (Table 1. 2) The pots in each treatment were spaced 2 feet apart; 30 days after planting date, the plants were inoculated with conidial suspension of *P. prunicola* with a concentration of 1000 conidia per ml using a surgeon's atomizer. Fungicide rates were calculated on the spray volume of 3,785 L/ ha.

For onion extract 453 grams of yellow onions were purchased from a grocery store and it was chopped into pieces, mixed with 1 liter of water and blended in a food processor the juice was extracted by filtering through a cheese cloth. The extract was applied to the plants directly using a spray bottle. EO water was sprayed a 7day intervals using a separate backpack sprayer. Remaining treatments were sprayed at 14 - day intervals from the 4 th week of May until the first week of September.

Evaluation of EO water for the management of Powdery mildew in a cherry orchard

Experiments were conducted in a commercial orchard located 25 km west of Quincy WA. The orchard comprised of sweet cherry trees cv. Lapin on Mazzard rootstock and the trees were of 17 year old. The spacing between the trees was 4.9 meters and spacing in between the rows was 5.5 meters. The trees have been treated with EO water from the year 2013. First 6 rows of the trees were treated with EO water once in a week and the remaining rows were treated with a standard fungicide in a 2-week spraying interval. Fungicides Myclobutanil (chemical name), trade name: Rally (Dow AgroSciences, Indianapolis, IN); 2. Triflumizole (Chemical name), Trade name: Pristine, BASF, Florham park, NJ) application started after first week of May. Standard fungicides (Myclobutanil, Triflumizole) were sprayed until the first week of July and EO water was sprayed until the middle of June. Electrostatic sprayer was used to treat the trees with EO water; these sprayers spray 190- 283 liters per acre in contrast to conventional sprayers, which spray 378 to 757 liters per acre.

Late season spray oil treatment

2% JMS stylet oil applied to the cherry trees in the nursery during the years 2012, 2013 and 2015. The experiment was designed as randomized complete block design with 4 replications. During the majority of the treatment window the trees were treated with alternations of DMI and or strobilurin fungicides. 2% JMS stylet oil was applied two times as a late season spray in late August and mid September. Each treatment had 10 plants; five were treated oil and 5 were left untreated as controls.

Disease evaluations in the nursery and green house

Disease severity and incidence were determined by selecting the first fully expanded leaves from the shoot apex of each plant. Disease severity was determined as the percent leaf area colonized by the powdery mildew on both sides of the leaves. Disease incidence was measured as the number of leaves with powdery mildew signs out of total leaves. For 2012 evaluations were carried out before the treatments and one week after the treatment once a month. In 2012 and 2014 the evaluations were carried out in a 2 -week interval, 1 week after the spray schedule (Table 1.3).

Orchard disease evaluations

In the cherry orchard the foliar evaluations were carried out during the cherry harvest season (middle of June) and continued until the middle of September. Trees that were treated with EO water and another 6 rows of trees that were treated with a standard fungicide were evaluated by randomly selecting a tree in the rows following W pattern. Disease severity was evaluated by randomly pruning the 5 terminal shoots using a tree pruner, and 5 shoots from the lower region of the canopy. Disease severity was measured as the percent leaf area colonized on each of ten leaves on each of 5 shoots from the upper and lower canopy, respectively.

Leaf collection for chasmothecia number and percent viability evaluation

At the end of powdery mildew epidemic leaves were collected from the nursery trees in 2012, 2013, 2015. Additional collections were made in the green house and orchard in the year 2015 to evaluate the effectiveness of different treatments on the chasmothecia production. In the nursery the leaves were collected from the middle 8 trees leaving first and last tree in each treatment. Fully expanded first 10 leaves were collected

from the trees and kept in the brown paper bags (16’ long 9.5’ wide). The leaves from a single treatment were pooled together and kept in a single bag. The bags were kept on lab benches and the leaves were shuffled everyday to let them dry; after 15 days the dried leaves were crushed by hand into a 1 L Mason jar and the blade unit from a blender vessel was attached to the jar. Each batch was comminuted for 30 seconds with pulsed power in order to produce a uniform sample. The blade unit and jar were cleaned with compressed air and Kim-wipes each time after processing each sample. Sample weights were recorded and processed samples stored in a refrigerator.

One gram of the ground leaf material from each sample was transferred to the top sieve of two-stacked sieves (USA Standard Testing Sieves, Mentor, OH, No.120 (0.125mm.), and 200 (0.074mm). Samples were washed twice for 30 seconds with a fan-shaped spray by squeezing the end of a 1cm diameter plastic tube. Each sample was moved around the sieve to invert leaf fragments between washes. The debris in the bottom sieve was backwashed and pushed to one side of the sieve and then collected in a 400 ml beaker by washing with about 100 ml of water. This volume was measured in a graduated cylinder and adjusted to a final volume of 100 ml by adding water. Samples were transferred back to the 400 ml beaker and the cylinder was washed with 50 ml water that was then transferred to the beaker. A stir bar was placed in beaker and the beaker was placed on a magna-stir apparatus where debris was kept in suspension. Ten milliliters of solution was measured quickly from the beaker (to avoid settling of the debris) and transferred to a Buchner funnel vacuum apparatus containing two 70 mm Whatman # 2 filter papers, which were transported to a dissection scope with forceps for observation. This procedure was repeated 4 times and the remaining solution was poured onto a single

filter paper to collect remaining chasmothecia. All equipment were washed with warm water and soap and allowed to air dry prior to processing subsequent samples. Five filter papers were visually examined with a dissection scope (10x), and the number of chasmothecia recorded. Ascospore viability was determined using the methods of Pearson and Gadoury (1987), Grove and Boal (1991) and Cortesi et al (1997).

Effect of different fungicidal compounds on chasmothecia viability

Chasmothecia were obtained on filter papers as described in earlier section; each filter paper contained approximately 1000 chasmothecia. Each chemical treatment trial had an untreated control, water control, and treatment set of filter papers (3 filter papers per set). The viability of chasmothecia was determined before treating the chasmothecia with different chemical and natural compounds. The chemical compounds were dissolved in water according to the field application rates (Table 7), and were applied on to the filter paper (with chasmothecia on them) using 50 ml atomizers until the filter paper is fully saturated.

Effect of fungicides on chasmothecia number and viability present on the stem

Cherry trees that were treated with standard fungicides chlorothalonil + Quinoxyfen, Fluopyram/ Trifloxystrobin and untreated trees were selected for tree washing experiment. Fifteen trees for each treatment and a total of 45 trees in the nursery were tagged with 3 different colored flagging tape 1-3/16" non-adhesive plastic ribbon at the end of fall (in the month of October during the years 2014 and 2015). These plants were dug in the month of November and stored in the storehouse. Beginning from the month of January of the following year, 10 trees were brought to the lab and washed as

described above. Each individual tree was cut above the grafted area and the upper part was removed and washed thoroughly using tap water. Chasmothecia from the stems were isolated onto a filter paper as described above.

Data analysis

Disease severity, incidence and the chasmothecia number and viability data from different treatments were subjected to Analysis of Variance (ANOVA) using general linear model (GLM) procedure, treatment means were separated by Fisher's Protected least significant difference (LSD) test at either $P<0.01$ or $P<0.05$. Analyses were performed using SAS version 8 (SAS Institute; Cary, NC)

Results

Evaluation of different fungicides in the nursery during the years 2012, 2014 and 2015

The disease pressure was high in the year 2015 compared to the years 2012 and 2014. During 2014 the disease onset occurred one month late compared to years 2012, 2013 and 2015. Epidemics of cherry powdery mildew in the nursery typically begin in mid- June and slow down in July due to high temperature (above 28 ^{o}C). Disease spread resumes when the weather cools and the epidemics increase exponentially from the 3rd week of July until the end of August. Chasmothecia formation typically begins in early September.

In the year 2012, in the middle of July the disease severity was low for the trees that were treated with EO water and the fungicide, Fluopyram/ Trifloxystrobin, the disease severity and incidence were significantly low ($P<0.05$) compared to the control and with Quinoxyfen treated plots. In late July Fluopyram/ Trifloxystrobin provided

control significantly different from control P<0.05. Disease severity and incidence of the plots that were treated with EO water was not different from that of Quinoxyfen treated plots (Tables 5 and 6). There was no significant difference observed in chasmothecia number and viability between different treatments (Table 7).

In 2014 and 2015, the trees treated with Actigard, *B. subtilis* and *B. pumilis* did not show significant difference in the disease severity, incidence and chasmothecia number when compared with untreated plots (P <0.05) (Tables 8,9,10,11). In 2014 *B. subtilis* alternated with Fluopyram/ Trifloxystrobin was found to be effective compared to the untreated plots. In the year 2015, EO water was applied once in a week and there was a significant reduction in the disease severity and incidence was observed. No significant difference was observed between EO water and standard fungicide (Tables 10 and11) treated plants when applied weekly. The number of chasmothecia formed were significantly lower for the EO water treated trees; Fluopyram/ Trifloxystrobin and Fluopyram/ Trifloxystrobin altered with *B. subtilis* (P<0.05) (Table 12). There was a significant reduction in the viability of chasmothecia was observed the trees that were treated with EO water when compared with control (P <0.05) (Table: 12).

Green house trial evaluations

Plants treated with *B. subtilis* showed highest disease severity and incidence compared to untreated control trees. Trees treated with EO water and Fluopyram/ Trifloxystrobin showed lower disease incidence and severity through out the epidemic. Trees treated with *B. pumilis,* Actigard and onion juice showed higher disease severity and incidence and were not effective in controlling the powdery mildew (Tables 15 and 16). There was a significant decrease in the chasmothecia number between the treated

and untreated trees (P<0.05) (Table 17). The number of chasmothecia were low due to the fact that *B. subtilis* and *B. pumilis* treated trees shed numerous leaves and only few leaves left that were not infected with powdery mildew. No significant difference was observed in the viability of chasmothecia between different treatments and untreated trees (Table 17).

Evaluation of EO water for the management of powdery mildew in a commercial cherry orchard

In the orchard, during the months of June and July, there was no significant difference in disease severity between trees that were treated with EO water and the rows that were treated with fungicides (Table 13). During the months of August and September, disease severity was significantly lower (p<0.05) in the trees that were treated with EO water compared to the trees that were treated with fungicides (Table: 13). The number of chasmothecia were significantly lower in the EO treated cherry tree leaves compared to fungicide treated tree leaves at P<0.05. The viability of chasmothecia was not significantly different between EO water treated trees and fungicide treated trees (Table 14).

Spray oil effect on chasmothecia number and viability

Results from the years 2012, 2013 and 2015 indicated that the trees when treated with 2% JMS Stylet Oil at the end of season, had significantly fewer chasmothecia (*P*<0.01) compared to untreated trees (Table 19). No significant difference (*P*<0.01) was observed in chasmothecia viability between the treated and untreated trees (Table 19).

Effect of different fungicidal compounds on chasmothecia viability

The effect of different natural sulfur containing compounds (such as onion extract, mustard oil) and other fungicidal compounds were tested their effects on chasmothecia viability. The average viability of chasmothecia before the treatment was determined as 17 % (the viability method as described by Grove, G.G., Boal, R.J., 1991). The viability of the chasmothecia was determined 1 week after treating the chasmothecia with different compounds (Table 18) and was significantly lower for the Onion extract and 95% ethyl alcohol treatments (P <0.05): other compounds were ineffective.

Effect of fungicides on the chasmothecia number and viability present on the stem

Results from 2014 and 2015 indicated that trees that were treated with fungicides had significantly (P<0.01) lower number of chasmothecia on the stems compared to untreated trees (Table 20). No significant difference (P<0.01) was observed in chasmothecia viability between the treated and untreated trees (Table 21).

Discussion

Close plant spacing and irrigation practices result in high relative humidity; the actively growing young tissue is also available through out the growing season, these conditions are suitable for the powdery mildew epidemic (Delp, 1954, Chellemi et al 1991, Willocquet et al 1996). Our experiments were designed to evaluate biofunigicides, EO water and narrow-range petroleum oils for managing the disease. Powdery mildew is generally managed using intensive synthetic fungicides regimens thus increasing the risk of the emergence of resistant pathogen strains. Fungicide resistance has been reported in different powdery mildew fungi (e.g. such as *Blumeria graminis, P.xanthii* and *E.neacator*; Bletter et al 1998, Brown et al 2002).

Although biofungicides were reportedly effective in managing the powdery mildew of different crops (Crist et al 2006, Moyer et al 2008, Romero et al 2004, Schilder et al 2002, Utkhede et al 2006), they were found to be ineffective in reducing the disease severity and chasmothecia formation of *P. prunicola* on sweet cherry nursery foliage. Actigard (acibenzolar-S- methyl) induces plant resistance against the diseases, and it was not effective in reducing the powdery mildew on cherry when applied alone. Low rates of Actigard when combined with standard fungicide programs greatly reduced the blue mold lesions and increased the yield of tobacco (LaMondia, J.A. 2008). Actigard in combination with synthetic fungicide or Actigard alternated with standard fungicides significantly reduced the disease severity of powdery mildew of cantaloupe (Matheron, M.E., Porchas, M. 2004). Actigard would have been effective when applied in combination with other standard fungicides. In our studies onion juice concentrate when combined with water was not effective in reducing powdery mildew severity in the greenhouse trials.

EO water applied weekly was effective in reducing powdery mildew severity and chasmothecia number on sweet cherry foliage in the studies that were conducted in the nursery, green house, and orchard. Biweekly application of EO water was not effective in controlling the powdery mildew. In the nursery and green house weekly applications were phytotoxic to leaf tissue (Figure 1). No phytotoxicity was observed in the orchard. This could be due to the use of electrostatic mist spray, when the EO water was sprayed using the backpack sprayer until runoff, the water remains on the margins of the leaf and the hypochlorus acid (HOCl) might be causing the leaf to burn by reacting with the

sunlight. EO water caused mild phytotoxicity in the green house grown gerbera daisy plants when sprayed two times a week (Mueller et al 2003).

Studies have shown that late-season applications of narrow-range petroleum oils has resulted in reductions in powdery mildew severity and survival inoculum in grapes (Avila et al 2010, Matheron et al 1998). Narrow range petroleum oils were effectively used against many plant pathogens, including sweet cherry powdery mildew fungi (Grove, G.G. and Boal, R., 1996, 1997 and 1998). In this study also, late season application of 2 % JMS Stylet oil reduced the chasmothecia number compared to the untreated trees. A study on grape powdery mildew (Hoffmann et al 2009) showed that fungicide applications decreased chasmothecia in the bark of grapevines. In our studies the number of chasmothecia present on the bark/ stem of cherry shoots were few and lower than numbers on untreated shoots. It was concluded that EO water could be used alternatively with synthetic fungicides sprays; more in depth evaluation should be carried out to study the phytotoxicity effects of EO water. Spray oils/ mineral oils are found to be effective in reducing the overwinter inoculum.

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Figure 1: Phytotoxicity symptoms observed on the cherry foliage when the trees were treated with EO water once in the Green house. Necrotic brown regions along the leaf margins were observed.

Table 1: Fungicides and biorational products used in the study for managing the powdery mildew.

Product name	Rate/ gallon	Active ingredient	Manufacturer
Luna sensation	0.37 ml	Fluopyram, Trifloxystrobin	Bayer, Research Triangle PK, NC
Serenade	1.14 gm	*Bacillus subtilis*	Bayer cropscience, Research Triangle PK, NC
Sonata	7.1 ml	*Bacillus pumilis*	Bayer cropscience, Research Triangle PK, NC
Actigard	0.38 g	Acibenzolar- S-methyl 50%	Syngenta, Greensboro, NC
Quintec	0.52 ml	Quinoxyfen	Dow AgroSciences, Indianapolis, IN
Stylet oil	76 ml	Paraffinic oil	JMS Flower Farms, Vero Beach, FL
Rally	0. 45ml	Myclobutanil	Dow AgroSciences, Indianapolis, IN
Pristine	0.41ml	Triflumizole	BASF, Florham park, NJ

Table 2: 2015 fungicide trial map in Quincy nursery, the experiment was designed as randomized complete block design (RCBD), random numbers for each replication were generated using Microsoft Excel program. Each treatment had 12 plants, first 2 plants and last 2 plants in the treatment plot were left as buffer plants and the middle 8 plants were sprayed until runoff.1st spray was on May 28th 2015, 2nd spray was on June 11th 2015, 3rd was on spray June 26 th 2015, 4th spray was on July 16th 2015, 5th spray was on August 6th 2015, 6 th spray was on August 27th 2015, 7 th spray was on September 3rd

Rep1 Row 183	Rep 2 Row 184	Rep 3 Row 185	Rep 4 Row 187
B.subtilis	EO water	*B.pumilis*	Fluopyram/ Trifloxystrobin
Control	Actigard	Fluopyram/ Trifloxystrobin	*B.subtilis*
B.pumilis	Fluopyram/ Trifloxystrobin	Control	EO water
Actigard	*B.subtilis*	Actigard	Control
EO water	Control	EO water	*B.pumilis*
Fluopyram/ Trifloxystrobin	*B.pumilis*	*B.subtilis*	Actigard

Table 3: 2015 Green house fungicide trial map, the experiment was designed as randomized complete block design, random numbers for each replication were generated using Microsoft Excel program. Each treatment had 5 plants, spraying was carried out until runoff. 1st spray was on May 27th 2015, 2nd spray was on June 10th, 3rd was on spray June 25th 2015, 4th spray was on July 15th 2015, 5th spray was on August 5th 2015, 6th spray was on August 26th 2015, 7th spray was on September 2nd

Rep1	Rep 2	Rep 3	Rep 4
B. subtilis	EO water	*B.pumilis*	Onion juice
Actigard	Onion juice	Fluopyram/ Trifloxystrobin	Actigard
B. pumilis	Fluopyram/ Trifloxystrobin	Control	EO water
Control	*B. subtilis*	Actigard	Control
EO water	Control	Onion juice	*B. pumilis*
Fluopyram/ Trifloxystrobin	*B. pumilis*	B. subtilis	Fluopyram/ Trifloxystrobin
Onion juice	Actigard	EO water	*B. subtilis*

Table 4: Disease evaluation dates that were conducted through the years 2012 to 2015

2012	2014	2015 Nursery	2015 Greenhouse	2015 Weber orchard
May 28	May 18	May 20	May 10	June 20
June 18	June 18	June 16	June 15	July 27
27July 27	July 18	June 20	June 19	August 20
August 27	August 5	July 18	July 16	September 5
	August 19	July 27	July 25	
	August 29	August 18	August 15	
		August 30	August 27	
		September 5	September 4	

Table: 5 Effect of EO water, Quinoline and SDHI (succinate dehydrogenase inhibitor) fungicides on the severity of sweet cherry powdery mildew in Quincy nursery 2012

Treatment[1]	**Disease Severity 2012**[2]		
	July 12	**July27**	**Aug 21**
EO water	0.4250[b3]	10.610[a]	8.985[a]
Quinoxyfen	2.5525[a]	10.945[a]	9.095[a]
Fluopyram/Trifloxy trobin	0.0875[b]	0.255[b]	2.53[b]
Untreated	2.6038[a]	14.66[a]	13.225[a]

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2] Percent leaf tissue affected by powdery mildew

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table: 5.1 ANOVA table for 2012 disease severity in Quincy cherry nursery.

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	3	21.8	7.26	70.82	<0.0001
Treat	3	459.3	153.1	16.78	0.0001
Treat	3	234.19	78.06	9.85	0.0015

Table: 6 Effect of EO water, Quinoline and SDHI (succinate dehydrogenase inhibitor) fungicides on the incidence of sweet cherry powdery mildew in Quincy nursery 2012

Treatment[1]	**Disease incidence 2012**[2]		
	July 12	**July27**	**Aug 21**
Untreated	0.84[a3]	1.00[a]	0.955[a]
Quinoxyfen	0.82[a]	0.975[a]	0.940[a]
EO water	0.60[b]	0.970a	0.89[a]
Fluopyram/ Trifloxystrobin	0.08[c]	0.1350[b]	0. 470[b]

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2] Proportion of leaves diseased

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 6.1: ANOVA table for 2012 disease incidence in Quincy cherry nursery.

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	3	1.48	0.494	130.65	<0.0001
Treat	3	2.15	0.717	483.73	<0.0001
Treat	3	0.639	0.213	19.69	<0.0001

Table 7: Effect of EO water, Quinoline and SDHI (succinate dehydrogenase inhibitor) fungicides on chasmothecia number in Quincy nursery 2012

Treatment[1]	**Chasmothecia number**[2]	**% Viable**
EO	55.10a[3]	12.5a
Quinoxyfen	33.05a	8.40a
Fluopyram/ Trifloxystrobin	35.80a	6.918a
Untreated	51.75a	14.30a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2] Number of chasmothecia obtained from 1 gram of ground leaf tissue

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 7.1: ANOVA table for 2012 chasmothecia number and viability on leaves from Quincy cherry nursery

Source	DF	Type III SS	Mean Square	F value	Pr>F
Treat	3	1481.5	493.85	0.37	0.77
Treat	3	142.73	47.57	1.54	0.25

Table 8: Effect of *Bacillus subtilis* (Serenade), SDHI +*Bacillus subtilis* altered with each other and SDHI alone (succinate dehydrogenase inhibitor) on disease severity in Quincy nursery 2014

Treatment[1]	**Disease Severity 2014**[3]			
	July 18	**Aug 5**	**Aug 19**	**Aug 29**
Serenade	0.015a	0.795a[4]	2.495a	5.650ab
Luna sensation	0.00a	0.00a	0.515b	1.955b
Luna sensation+serenade[2]	0.005a	0.16a	0.117b	7.345ab
Untreated	0.015a	0.0100a	3.875a	11.745a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2] SDHI +*Bacillus subtilis* altered every 14 days

[3] Percent leaf tissue affected by powdery mildew

[4]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table: 8.1 ANOVA table for 2014 disease severity in Quincy cherry nursery

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	3	0.00	0.00	1.08	0.39
Treat	3	1.70	0.57	0.99	0.43
Treat	3	37.05	12.35	2.64	0.10
Treat	3	197.93	65.98	2.87	0.08

Table 9: Effect of *Bacillus subtilis* (Serenade), SDHI +*Bacillus subtilis* altered with each

Treatment[1]	**Disease Incidence 2014**[3]			
	July 18	**Aug 5**	**Aug 19**	**Aug 29**
Serenade	0.015a[4]	0.04a	0.585ba	0.9750a
Luna sensation	0.00a	0.00a	0.115c	0.650b
Luna sensation + serenade[2]	0.005a	0.030a	0.315bc	0.975a
Untreated	0.015a	0.0100a	0.825a	0.925ba

other and SDHI alone (succinate dehydrogenase inhibitor) on disease Incidence in Quincy nursery 2014

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2] SDHI +*Bacillus subtilis* altered every 14 days

[3] Proportion of leaves diseased

[4]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 9.1: ANOVA table for 2014 disease incidence in Quincy cherry nursery.

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	3	0.00	0.00	1.08	0.39
Treat	3	0.00	0.00	1.54	0.26
Treat	3	1.16	0.39	8.04	0.00
Treat	3	0.29	0.10	2.82	0.08

Table 10: Effect of *Bacillus subtilis* (Serenade), *Bacillus pumilis* (sonata), EO water, plant activator (Actigard) and SDHI on disease severity in Quincy nursery 2015

Treatment[1]	**Disease severity 2015**[2]				
	July 18	**July 30**	**Aug 14**	**Aug 24**	**Sep 3**
EO water	0.130a[3]	0.7ab	20.46c	20.80b	29.9c
Serenade	0.22a	0.87ab	39.55a	51.15a	58.15b
Luna sensation	0.17a	0.49b	30.95b	26.42b	33.46c
Actigard	0.29a	0.90a	39.55a	49.40a	61.70ab
Sonata	0.17a	0.89a	40.59a	48.85a	59.15b
Untreated	0.22	1.02a	42.25a	54.35a	68.5a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2]Percent leaf tissue affected by powdery mildew

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 10.1: ANOVA table for 2014 disease severity in Quincy cherry nursery

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	5	0.062	0.012	0.740	0.602
Treat	5	0.707	0.141	2.150	0.106
Treat	5	1404.637	280.927	17.240	<.0001
Treat	5	4119.708	823.942	22.480	<.0001
Treat	5	5148.106	1029.621	46.470	<0.0001

Table 11: Effect of *Bacillus subtilis* (Serenade), *Bacillus pumilis* (sonata), EO water, plant activator (Actigard) and SDHI on disease incidence in Quincy nursery 2015

Treatment[1]	**Disease Incidence 2015** [2]				
	July 18	**July 30**	**Aug 14**	**Aug 24**	**Sep 3**
EO water	0.170a[3]	0.54bc	1.00a	1.00a	1.00a
Actigard	0.21a	0.74ba	1.00a	1.00a	1.00a
Serenade	0.18a	0.71ba	1.00a	1.00a	1.00a
Sonata	0.22a	0.630ba	1.00a	1.00a	1.00a
Luna sensation	0.17a	0.41c	1.00a	1.00a	1.00a
Untreated	0.22a	0.69ba	1.00a	1.00a	1.00a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2] Proportion of leaves diseased

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table11.1: ANOVA table for 2015 disease incidence in Quincy cherry nursery

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	5	0.01013	0.00203	0.15000	0.97840
Treat	5	0.31200	0.06240	3.64000	0.01890
Treat	5	0.00000	0.00000		
Treat	5	0.00000	0.00000		
Treat	5	0.00000	0.00000		

Table 12: Effect of *Bacillus subtilis* (Serenade), *Bacillus pumilis* (sonata), EO water, plant activator (Actigard) and SDHI on chasmothecia number in Quincy nursery 2015

Treatment[1]	**Chasmothecia Number**[2]	**% Chasmothecia viable**
EO water	210b[3]	4.5c
Serenade	1149ab	18.25ab
Luna sensation	446b	10.75bc
Actigard	3789a	16abc
Sonata	1106 ab	24.25a
Luna sensation +Serenade	459b	10.25bc
Untreated	1428ab	28.25a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2] Number of chasmothecia obtained from 1 gram of ground leaf tissue

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 12.1: ANOVA table for 2012 chasmothecia number and viability on leaves from Quincy cherry nursery

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	6	36539417.5	6089902.9	1.71	0.17
Treat	6	1664.2	277.4	3.49	0.02

Table 13: Effect of EO water, and fungicides (Pristine/ Rally) on disease severity in Quincy orchard in the year 2015

Treatment[1]	**Disease severity**[2]			
	June 16	**July16**	**Aug16**	**Sept16**
EO water	5.428a[3]	10.922a	10.246b	6.306b
Fungicide	6.068a	7.832a	23.071a	12.934a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew (from mid May)

[2]Percent leaf tissue affected by powdery mildew

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 13.1: ANOVA table for Quincy orchard disease severity in 2015

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	1	4.096	4.096	0.37	0.5442
Treat	1	95.481	95.481	3.54	0.0674
Treat	1	1644.81	1644.81	66.21	<.0001
Treat	1	439.30	439.30	32.18	<.0001

Table: 10 Weber orchard chasmothecia number and viability

Table 14: Effect of EO water, and fungicides (Pristine/ Rally) on chasmothecia number in Quincy orchard in the year 2015

Treatment[1]	**Chasmothecia number**[2]	**% Viable**
EO	33.12b[3]	40.337a
Fungicide	157.44a	38.814a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew(mid May)

[2] Number of chasmothecia obtained from 1 gram of ground leaf tissue

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 14.1: ANOVA table for 2012 chasmothecia number and viability on leaves from Quincy cherry nursery

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	1	154554.6	154554.6	32.3	<0.0001
Treat	1	23.2	23.2	0.1	0.8

Table: 15 Effect of *Bacillus subtilis* (Serenade), *Bacillus pumilis* (sonata), EO water, plant activator (Actigard) and SDHI on disease severity in the green house experiment conducted in the year 2015

Treatment[1]	**Disease severity Green house 2015**[2]					
	June 5	**June20**	**July 18**	**Aug 5**	**Aug20**	**Aug28**
Actigard	0.00c [3]	1.33bc	7.907ab	13.57bc	25.58bc	18.09bc
Sonata	0.14abc	3.13b	9.48ab	22.58b	17.73c	19.18bc
Serenade	0.270a	5.72a	15.14a	28.18ab	34.68ab	26.49ab
Onion extract	0.108abc	1.74bc	8.72ab	11.79bc	15.34dc	10.23dc
Luna sensation	0.010bc	0.00c	1.572b	1.072c	1.08d	0.802d
EO water	0.00c	0.30c	0.38b	1.340c	0.990d	0.630d
Untreated	0.21ab	1.83bc	17.672a	42.802a	44.26a	38.162a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2]Percent leaf tissue affected by powdery mildew

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 15.1: ANOVA table for green house disease severity in 2015

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Treat	6	0.35	0.06	2.41	0.0525
Treat	6	112.75	18.79	6.4	0.0002
Treat	6	1216.20	202.70	5	0.0014
Treat	6	6794.62	1132.44	6.93	0.0001
Treat	6	7907.82	1317.97	8.65	<.0001
Treat	6	5579.20	929.87	7.69	<.0001

Table 16: Effect of *Bacillus subtilis* (Serenade), *Bacillus pumilis* (sonata), EO water, plant activator (Actigard) and SDHI on disease incidence in green house 2015

Treatment	**Disease Incidence Green house 2015**					
	June 5	**June 20**	**July 18**	**Aug 5**	**Aug20**	**Aug28**
Actigard	0.00b	0.24b	0.61a	0.83a	0.83a	0.87a
Sonata	0.1ba	0.52a	0.68a	0.87a	0.93a	0.88a
Serenade	0.23a	0.58a	0.78a	0.81a	0.95a	0.92a
Onion extract	0.11ba	0.56a	0.86a	0.97a	0.95a	0.96a
Luna sensation	0.010b	0.00b	0.34b	0.35b	0.29b	0.37b
EO water	0.020b	0.090b	0.14b	0.390b	0.44b	0.26b
Untreated	0.17a	0.67a	0.86a	0.92a	0.92a	0.99a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew

[2] Proportion of leaves diseased

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table16.1: ANOVA table for green house disease incidence in 2015

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	6	0.22942857	0.0382381	3.65	0.008
Treat	6	2.121	0.3535	9.25	<0.0001
Treat	6	2.263	0.37716667	9.42	<.0001
Treat	6	1.94785714	0.32464286	11.87	<.0001
Treat	6	2.27442857	0.37907143	11.56	<0.0001
Treat	6	8.65495238	1.44249206	46.19	<0.0001
Treat	6	2.732	0.45533333	21.32	<0.0001

Table 17: Effect of EO water, and biofungicides on chasmothecia number in green house in the year 2015

Treatment[1]	**Chasmothecia number**[2]	**% Viable**
Actigard	75.2b[3]	5.63a
Sonata	49.2b	4.74a
Serenade	126.4b	7.4a
Luna sensation	1.1b	6.66a
Onion extract	103.5b	13.88a
EO water	4.5b	9.22a
Untreated	649.7a	15a

[1]Sprays were applied at 14-day intervals beginning at the first signs of powdery mildew (mid May)

[2] Number of chasmothecia obtained on filter paper from 1 gram of ground leaf tissue

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 17.1: ANOVA table for 2015 chasmothecia number and viability on leaves from greenhouse

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	6	1556519.063	259419.844	8.97	<.0001
Treat	6	485.6791867	80.9465311	1.15	0.3585

Table 18: Effect of chemical compounds and biorational products on chasmothecia viability

	Rate	Treatment[1]	% Viable after treatment[2]
1	-	Onion juice	1h[3]
2	-	Mustard oil	9.8f
3	0.1g/50ml	Copper sulfate	13.2cde
4	0.1g/50ml	Copper hydroxide	12.5 def
5		Stylet oil 2%	10.4f
6	0.2g/50ml	Potassium monophoshate	12.4def
7		0.5% bleach	11.8 ef
8	-	95% EtOH	1.4h
9	-	75% EtOH	4.4g
10	-	50% EtOH	11.1ef
11	-	10% EtOH	16.4b
12		Sulfur	15.8a
13	-	Water	19.8a
14	-	Untreated	14.7cbd

[1] Chasmothecia were treated with various products
[2] Viability was determined by picking 100 chasmothecia from the filter paper, after 7 days of treatment
[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 18.1: ANOVA table for chasmothecia viability on leaves from green house

Source	DF	Type IIISS	Mean Square	F value	Pr>F
Treat	13	3905.15	300.39	31.87	<.0001

Table 19: Effect of 2% JMS stylet oil on chasmothecia number and viability during the years 2012, 13 and 15.

Chasmothecia number[1]			Viability[2]	
Year	With oil	Without oil	With oil	Without oil
2012	28.02b[3]	221.5a	9.82a	10.87a
2013	75.82b	346.71a	7.7a	10.5a
2015	909.645b	1383.26a	17.31a	19.02a

[1] Average number of chasmothecia obtained on filter paper from 1 gram of ground leaf tissue

[2] Viability was determined by picking 100 chasmothecia from the filter paper, after 7 days of treatment

[3]Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.05$

Table 19.1: ANOVA table for effect of 2% JMS stylet oil on chasmothecia number

Year	Source	DF	Type IIISS	Mean Square	F value	Pr>F
2012	Treat	1	774499.1	774499.1	30.7	0.0
2013	Treat	1	660427.1	660427.1	31.2	<.0001
2015	Treat	1	4441373.4	4441373.4	29.8	<.0001

Table 19.2: ANOVA table for effect of 2% JMS stylet oil oil on chasmothecia viability

Year	Source	DF	Type IIISS	Mean Square	F value	Pr>F
2012	Treat	1	0.1621	0.1621	0.0100	0.9467
2013	Treat	1	70.5180	70.5180	3.6600	0.0620
2015	Treat	1	57.8698	57.8698	1.3300	0.2521

Table 20: Effect of two different fungicides on chasmothecia number on cherry shoots, Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.01$

Effect of fungicides on chasmothecia number (on stems)		
	Year	
	2014[1]	2015[1]
Treatment		
Luna sensations	18.25b	53.1b
Quintec + Fontelis	49.7b	187.45b
Untreated	109.78a	358.7a

[1] Five trees of each treatment were washed each year beginning from the month of January and continued until June and the average number of chasmothecia were determined by washing the trees

Table 20.1: ANOVA table for effect fungicides on chasmothecia number on cherry shoots

Source	DF	Type III SS	MS	F value	Pr>F
Treat	2	129754.5575	64877.2788	12.48	<.0001
Treat	2	543893.8423	271946.9212	10.6	0.0002

Table 21: Effect of two different fungicides on chasmothecia viability on cherry shoots, Means followed by the same letter are not significantly different according to GLM procedure LSD test at $p<0.01$

Effect of fungicides on chasmothecia viability (stems)		
	Year	
	2014[1]	2015[1]
Treatment		
Luna sensations	0.05a	3a
Quintec+ Fontelis	0.7a	2.35a
Untreated	2.02a	6a

[1] Five trees of each treatment were washed each year beginning from the month of January and continued until June and the average number of viable chasmothecia were determined by washing the trees

Table 21.1: ANOVA table for effect fungicides on chasmothecia viability on cherry shoots

Source	DF	Type III SS	MS	F value	Pr>F
Treat	2	60.4575	30.22875	2.73	0.0718
Treat	2	109.7290698	54.8645349	1.66	0.2026

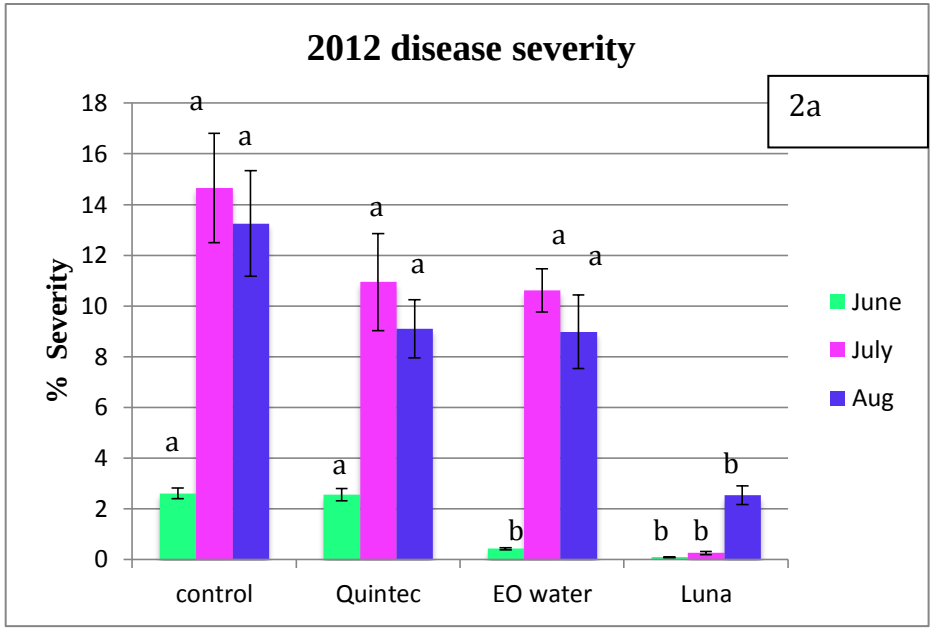
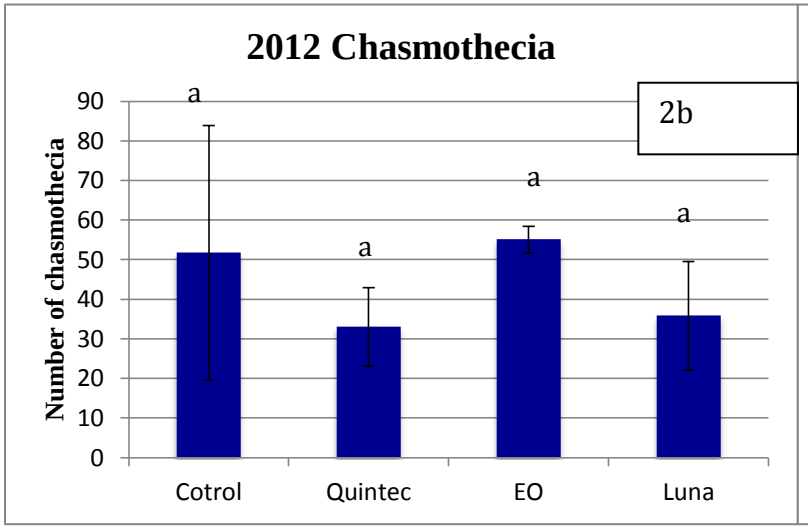
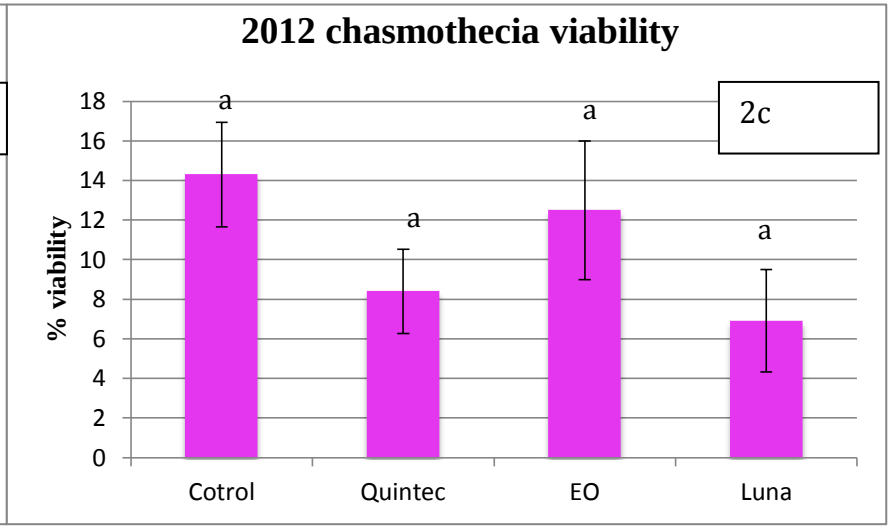


Figure 2: Effocacy of EO water for the year 2012 in Quincy nursery, figure 2a shows disease severity data; figure 2b shows chasmothecia number data and figure 2c shows chasmothecia viability data.





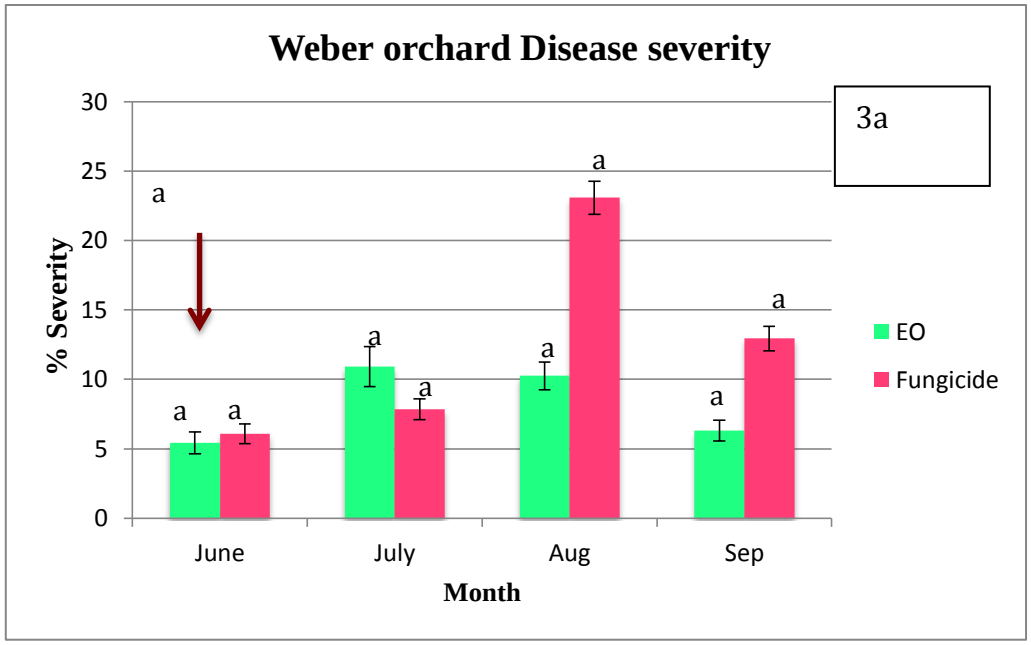
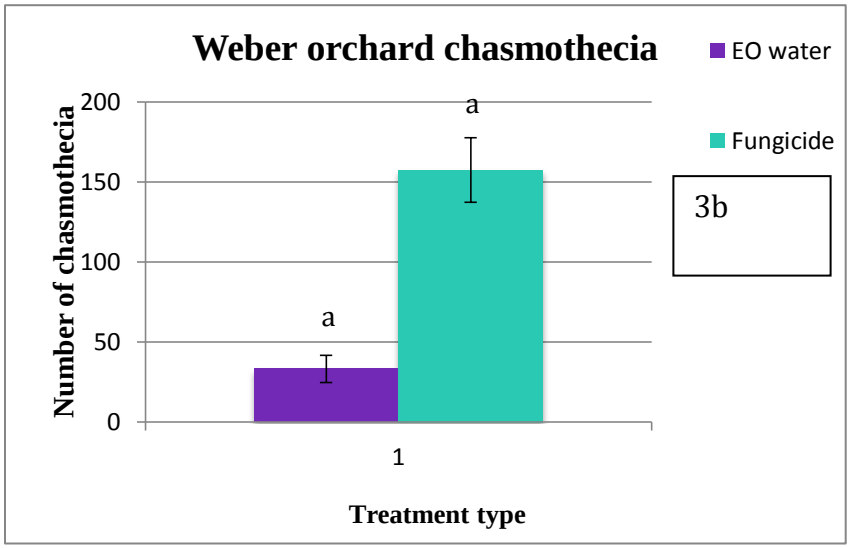
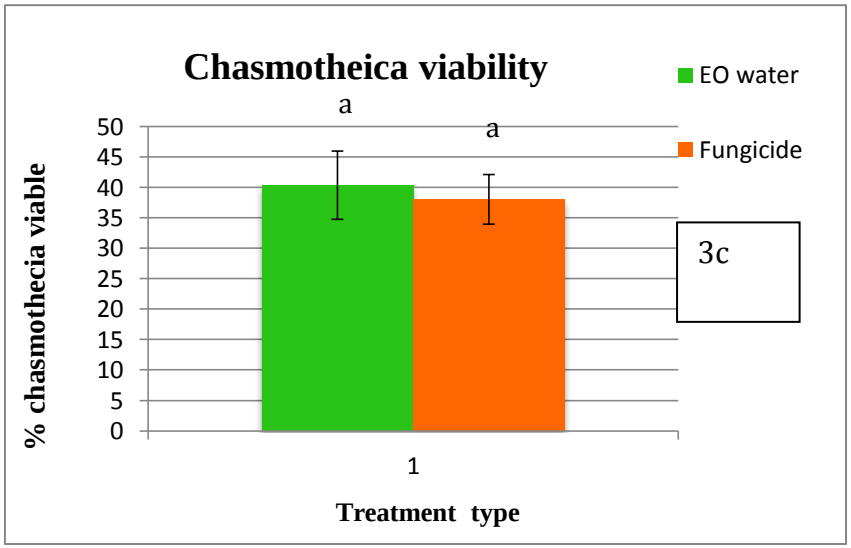


Figure 3: Disease severity data for the year 2015 in Quincy orchard, figure 3a shows disease severity data; figure 3b shows chasmothecia number data and figure 3c shows chasmothecia viability data.





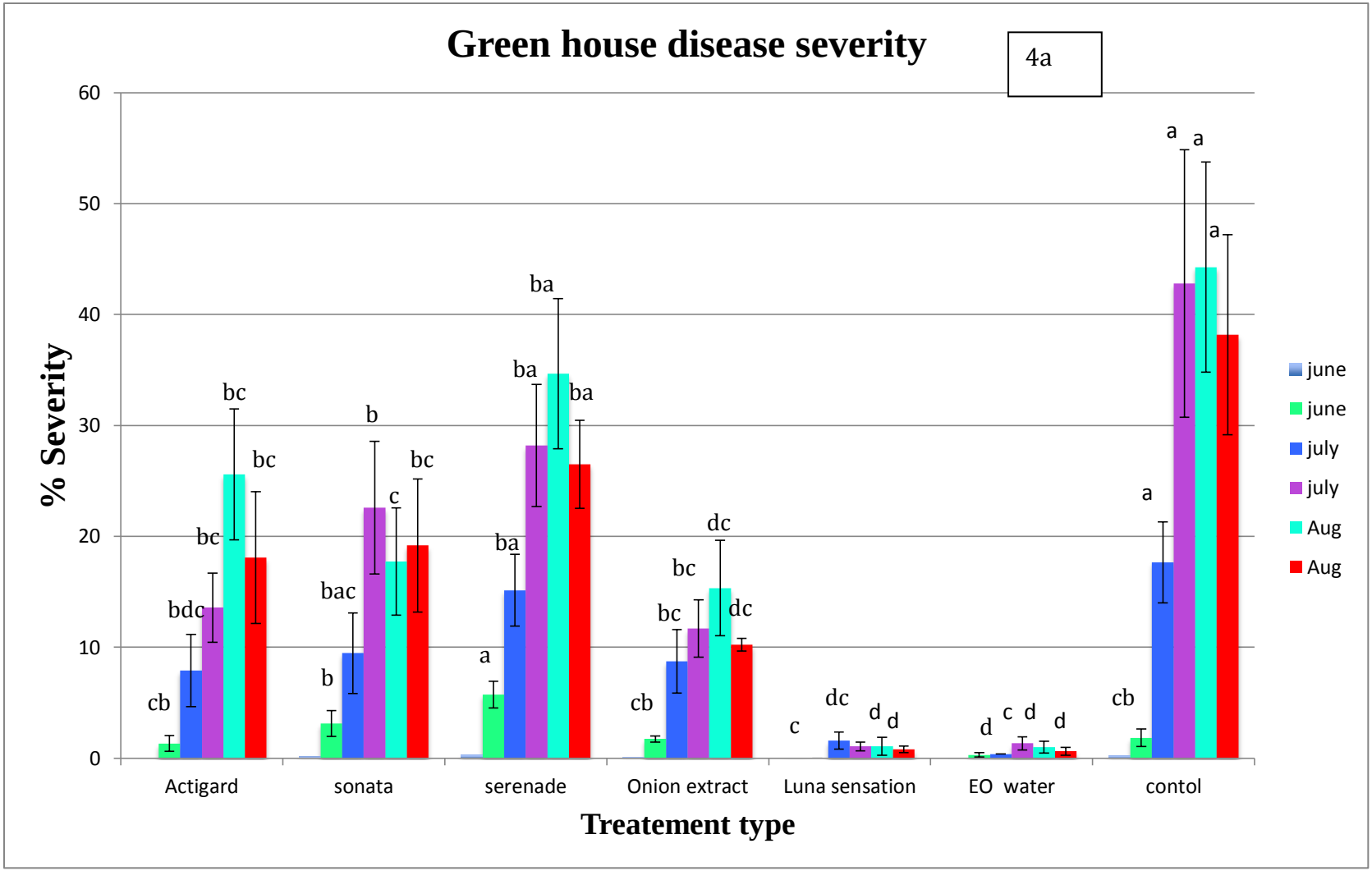
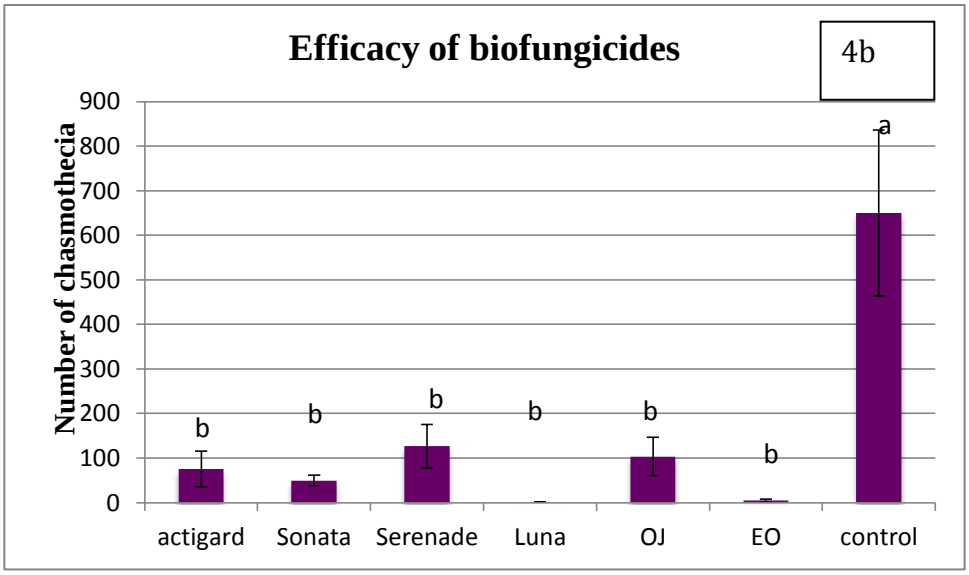


Figure 4a: Disease severity data of green house growen sweet cherry trees in the year 2015



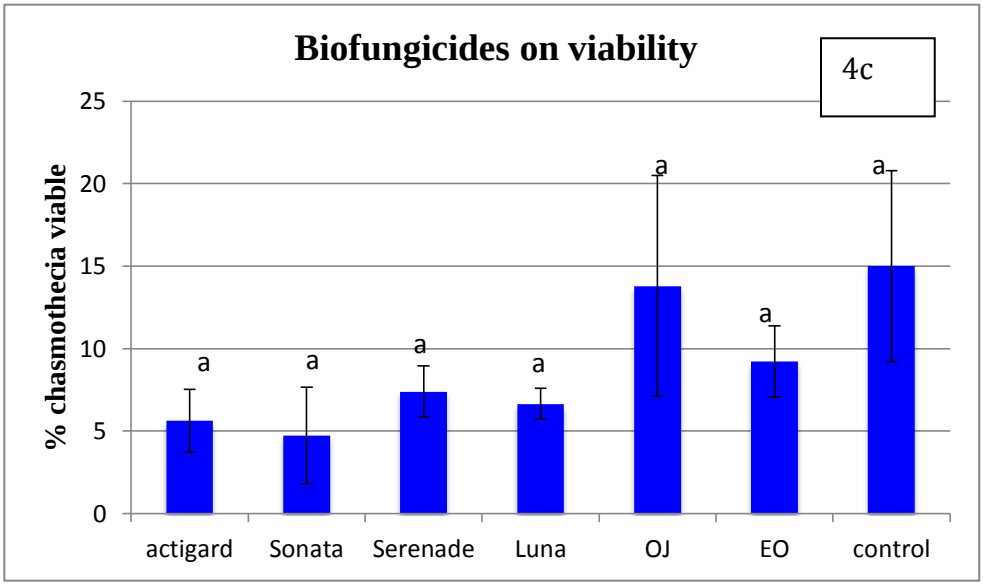
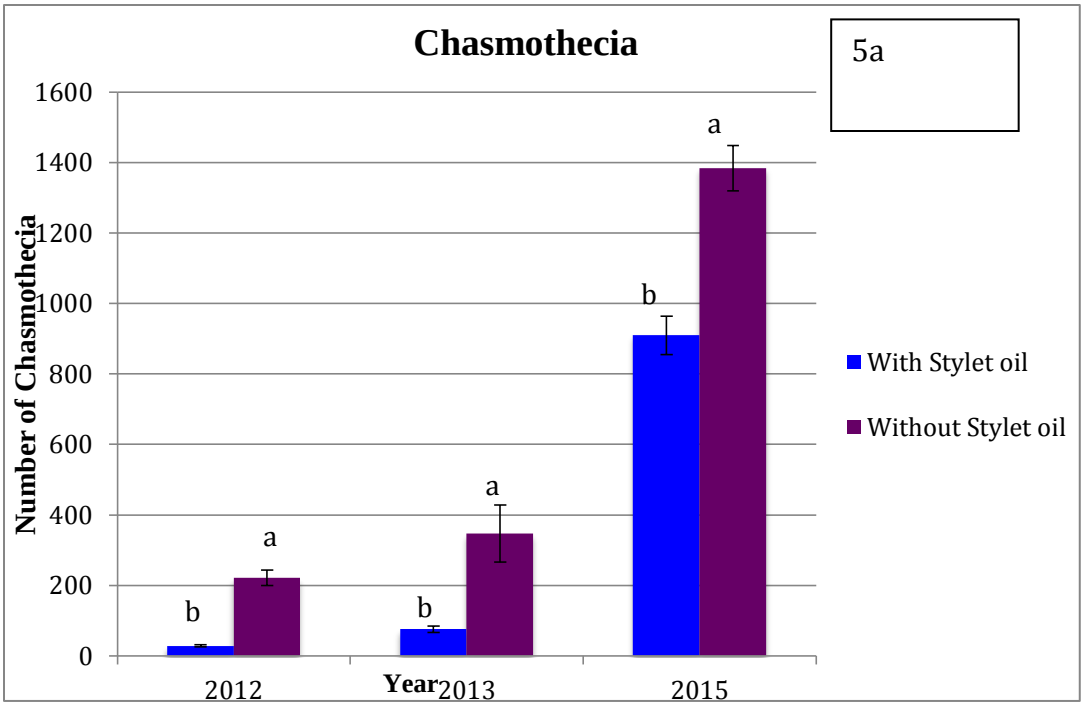


Figure 4b: Effect various treatments on chasmothecia number in green house grown cherry trees, 4c: Effect various treatments on chasmothecia viability in green house grown cherry trees,



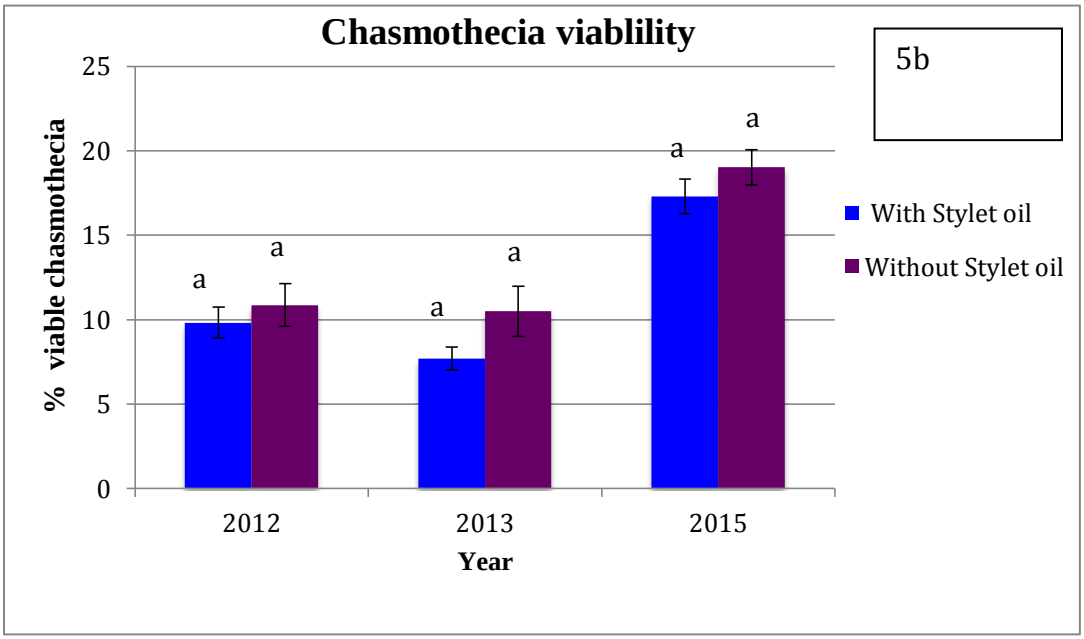


Figure 5a: Effect of styet oil in chasmothecia formation during the years 2013, 2014 and 2015, 5b: Effect of styet oil in chasmothecia number during the years 2013, 2014 and 2015

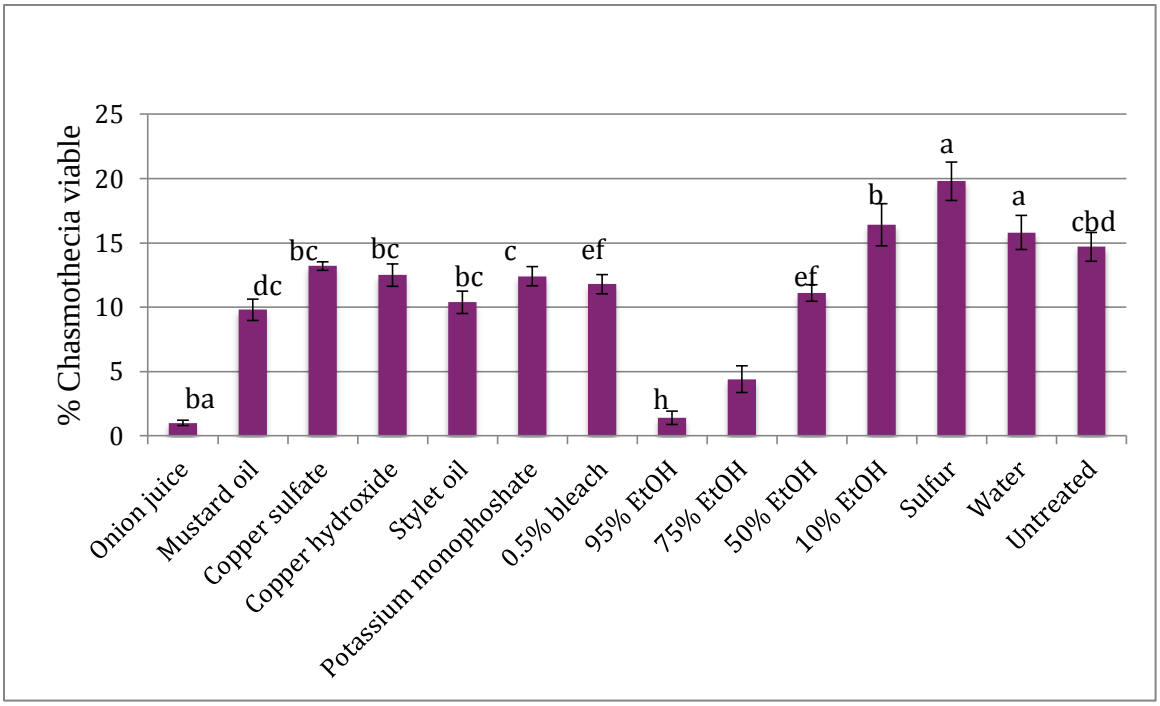


Figure 6: Effect of various chemical compounds on chasmothecia viability