ASSESSMENT OF TOMATO AND LETTUCE DISEASES IN ORGANICALLY
MANAGED HIGH TUNNEL CROPPING SYSTEMS IN WESTERN
WASHINGTON AND ISOLATION OF SOIL MICROBES
POTENTIALLY CAPABLE OF BIODEGRADING
AGRICULTURAL MULCHES

By

MARIANNE POWELL

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

MASTER OF SCIENCE IN PLANT PATHOLOGY

WASHINGTON STATE UNIVERSITY
Department of Plant Pathology

DECEMBER 2012
To the Faculty of Washington State University:

The members of the Committee appointed to examine the Master’s Thesis of MARIANNE POWELL find it satisfactory and recommend that it be accepted.

__________________________
Debra A. Inglis, Ph.D., Chair

__________________________
Carol Miles, Ph.D.

__________________________
Marion Brodhagen, Ph.D.

__________________________
Linda S. Thomashow, Ph.D.
Professional

I would like to thank my advisor, Dr. Debra Ann Inglis, for the opportunities extended to me while under her guidance. I developed critical evaluation and writing skills under her tutelage, which empowers me as a scientist. Her enthusiasm in my discoveries and her encouragement and patience during difficult times was greatly appreciated. I would also like to thank all my committee members; Dr. Carol Miles, Dr. Marion Brodhagen, and Dr. Linda Thomashow, for their contributions and guidance which helped me realize my potential, keep me focused, and introduce me to new sources of information. Dr. Miles and her lab personnel contributed immensely to my field research studies and I am grateful for their dedication and services which made this project possible. Their cheerful “can do” attitude is admirable. Dr. Miles was a great resource and her desire to help me succeed is greatly appreciated. It was a great pleasure to work in Dr. Brodhagen’s laboratory. I admire her acute attention to detail and will forever be grateful for the laboratory skills I learned from her. I also extend gratitude to Babette Gundersen, who was instrumental to the success of my project while I was on campus and in Corvallis. I appreciate her kindness and advice. I would also like to thank Dr. Brenda Schroeder and Dr. Tim Paultiz for the use of their laboratories while I was in Pullman. I would especially like to thank Dr. Schroeder and Dr. Jodi Humann for their assistance in identifying and testing pathogenicity of bacterial isolates. I am grateful to the Department of Plant Pathology staff in Pullman and to the staff at the Mount Vernon NWREC who have come to my aid, helping me navigate WECN, make travel arrangements, and provide me with space and tools to help me complete my experiments.
Personal

I am most grateful for my biggest support, Morris D. Wainwright, whose friendship and love has helped me to believe in myself and strive to achieve goals. I would also like to thank my sisters and brothers who have encouraged me throughout my life and have been a source of comfort. Finally, I would like to thank old and new friends who have helped me to relax and have fun while also understanding the hectic schedule of a student.
ASSESSMENT OF TOMATO AND LETTUCE DISEASES IN ORGANICALLY
MANAGED HIGH TUNNEL CROPPING SYSTEMS IN WESTERN
WASHINGTON AND ISOLATION OF SOIL MICROBES
POTENTIALLY CAPABLE OF BIODEGRADING
AGRICULTURAL MULCHES

Abstract
by Marianne Powell, M.S.
Washington State University
December 2012

Chair: Debra Ann Inglis

High tunnels (HT) offer an alternative cropping system for specialty crops grown in western Washington, where growing degree days are limited and strong demand for locally-produced foods may offset the added expense of protected cultivation. Environmental conditions, and lettuce diseases on six cultivars representing three market classes (Boston, Leaf, and Romaine), were evaluated in HT and open field (OF) production systems from 2010 to 2012. Tomato diseases on ‘Celebrity’ grown with biodegradable mulches (BDM) under these two systems also were assessed. Mulch study treatments included commercial, biodegradable products: BioAgri (BA), BioTelo (BT), and WeedGuardPlus (WG); one experimental spunbond-poly(lactic) acid product; black polyethylene (BP) standard; and, a non-mulched control. For the lettuce trial, relative humidity between the production systems was similar, but average soil and air temperatures were greater in the HT compared to OF all three years. Gray mold, caused by *Botrytis cinerea*, was significantly higher in Romaine versus other types. Gray mold was more severe in HT compared to OF but these effects were cultivar-dependent. Lettuce drop caused by
Sclerotinia sclerotiorum was observed in all lettuce cultivars all years. In 2012, incidence of lettuce drop was significantly higher and sclerotia more numerous in harvested heads from HT compared to OF. Verticillium wilt was noted on lettuce each year and incidence increased annually, from 0.2% to 2% to 14%. V. tricorpus was isolated from symptomatic heads each year and all isolates were pathogenic in repeated greenhouse trials. This is the first report of V. tricorpus causing Verticillium wilt on lettuce in Washington. ‘Green Star’ performed well in both systems, with reduced incidence of gray mold, lettuce drop, and Verticillium wilt compared to other cultivars. For tomato, late blight was significantly lower in HT compared to OF, but severity of physiological leaf roll (PLR) was significantly higher in HT compared to OF. PLR was also significantly higher with BA, BT, and BP mulches. Root diseases were not affected by mulch treatment, and not observed on tomato plants. Methods for isolating native fungi that colonize agricultural mulches in soil were refined, and three isolates each were acquired from BA, BT, and WG.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1. GENERAL LITERATURE REVIEW ON HIGH TUNNELS AND CROP</td>
<td></td>
</tr>
<tr>
<td>Overview of high tunnels</td>
<td>1</td>
</tr>
<tr>
<td>High tunnels and the environment</td>
<td>2</td>
</tr>
<tr>
<td>High tunnels and plant diseases</td>
<td>5</td>
</tr>
<tr>
<td>Overview of mulches</td>
<td>7</td>
</tr>
<tr>
<td>Biodegradable mulches</td>
<td>9</td>
</tr>
<tr>
<td>Mulches and plant diseases</td>
<td>12</td>
</tr>
<tr>
<td>Common diseases on tomato in western Washington</td>
<td>13</td>
</tr>
<tr>
<td>Late blight</td>
<td>13</td>
</tr>
<tr>
<td>Gray mold</td>
<td>15</td>
</tr>
<tr>
<td>Tomato pith necrosis</td>
<td>17</td>
</tr>
<tr>
<td>Common diseases on lettuce in western Washington</td>
<td>18</td>
</tr>
<tr>
<td>Gray mold and lettuce drop</td>
<td>18</td>
</tr>
<tr>
<td>Verticillium wilt</td>
<td>22</td>
</tr>
<tr>
<td>Experimental high tunnel and mulch trials</td>
<td>25</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>28</td>
</tr>
</tbody>
</table>
2. COMPARISON OF DISEASE OUTBREAKS IN ORGANICALLY-MANAGED TOMATOES GROWN WITH DEGRADABLE MULCHES IN HIGH TUNNELS VS. OPEN FIELD SETTINGS IN WESTERN WASHINGTON

Introduction ................................................................. 43
Materials and Methods ..................................................... 47
Results ........................................................................... 54
Discussion ....................................................................... 61
Literature Cited ................................................................. 66

3. COMPARISON OF DISEASE OUTBREAKS ON LETTUCE CULTIVARS GROWN ORGANICALLY WITH POLYETHYLENE MULCH IN HIGH TUNNEL VS. OPEN FIELD SETTINGS IN WESTERN WASHINGTON

Abstract ........................................................................ 89
Introduction ................................................................... 90
Materials and Methods ..................................................... 96
Results ........................................................................... 105
Discussion ....................................................................... 115
Literature Cited ................................................................. 125
APPENDICES

A. PROCEDURES FOR ISOLATING NATIVE SOIL MICROORGANISMS FROM BIODEGRADABLE PLASTIC FILMS…………………………………….. 153

B. SURVIVAL OF SCLEROTINIA SCLEROTIORUM IN SOIL AS AFFECTED BY BIODEGRADABLE MULCHES……………….. 170

C. PRELIMINARY STUDY ON SOIL SOLARIZATION TO REDUCE SOILBORNE INOCULUM OF VERTICILLIUM……………………….. 175

D. FIRST REPORT OF VERTICILLIUM WILT ON LETTUCE (Lactuca sativa L.) IN WASHINGTON CAUSED BY Verticillium tricorpus L…………………………………………………………….. 180
LIST OF TABLES

1. Disease and pest control application rates and schedules used on tomatoes grown under high tunnel and open field production systems in experimental trials near Mount Vernon, WA………………………………………………………… 73
2. Average soil and air temperatures, mid-May to early-October, for five agricultural mulches and bare ground with tomato cultivar Celebrity in an experimental field trial Comparing high tunnel and open field production near Mount Vernon, WA………74
3. Area under disease progress curve (AUDPC) values in 2010, and mean number of late blight lesions in 2011 and 2012 for tomato cultivar Celebrity grown with five agricultural mulches and bare ground in experimental field trials comparing high tunnel and open field production near Mount Vernon, WA…………………………… 75
4. Relative area under disease progress curve (RAUDPC) values for physiological leaf roll on tomato as affected by five agricultural mulches and bare ground in experimental field trials comparing high tunnel and open field production systems near Mount Vernon, WA in 2010, 2011, and 2012…………………………………… 76
5. Comparisons of diseased fruit of tomato cultivar Celebrity at harvest for five agricultural mulches and bare ground in experimental field trials on high tunnel and open field production systems near Mount Vernon, WA in 2011 and 2012…..77
6. Yield comparisons for tomato cultivar Celebrity grown under five agricultural mulches and bare ground in experimental field trials on high tunnel and open field production systems near Mount Vernon, WA in 2010, 2011, and 2012………… 78
7. Lettuce type and cultivar planted during field trials evaluating lettuce cultivars grown in high tunnel and open field plots near Mount Vernon, WA ......................... 137
8. Dates for planting lettuce, constructing high tunnels, and fertigating during experimental field trials evaluating lettuce cultivars grown in high tunnels and open fields near Mount Vernon, WA ................................. 138

9. Comparison of disease onset and time to harvest for lettuce cultivars in high tunnel and open field production systems near Mount Vernon, WA based on days after transplanting. Experimental field trials were conducted during the spring, from 2010 through 2012................................................................. 139

10. Incidence of disease on harvested lettuce heads by production system and cultivar In experimental field trials near Mount Vernon, WA in 2010, 2011, and 2012...... 140

11. Recovery, viability, and size of sclerotia produced in lettuce tissue infected by Sclerotinia sclerotiorum from harvested lettuce plants grown in experimental plots near Mount Vernon, WA in 2012................................................................. 141
LIST OF FIGURES

1. Lettuce in raised beds, mulched with black polyethylene, transplanted by hand with 31 cm spacing between plants in two staggered rows and A, seedlings inside a high tunnel plot and B, mature heads in open field……………………42

2. Comparison of soil temperatures in non-raised beds covered with five agricultural mulches and a bare ground control used for growing tomato cultivar Celebrity in experimental field trials on high tunnel (HT) and open field (OF) production systems near Mount Vernon, WA. 1A, 2010 HT; 1B 2010 OF; 2A, 2011 HT; 2B, 2011 OF; 3A, 2012 HT; 3B, 2012 OF………………………………………………… 79

3. Air temperature measured in the center of one high tunnel (HT) and one open field (OF) main plot for tomato cultivar Celebrity grown near Mount Vernon, WA 2010, 2011, and 2012. ……………………………………………………………………… 82

4. Severity of late blight on tomato cultivar Celebrity grown under high tunnel and open field production systems in a field experiment near Mount Vernon, WA, 2010. A, late blight severity (1% of plot affected) of tomato plants grown under a high tunnel compared to B, severe late blight (80% of plot affected) in an open field plot…………………………………………………………………… 83

5. Rating scale used to assess differences in the severity of physiological leaf roll (PLR) on tomato among five agricultural mulches and a bare ground control in an experiment comparing high tunnel and open field production near Mount Vernon, WA, 2010, 2011, and 2012……………………………………………………… 84

6. Tomato pith necrosis as observed in an experimental field trial near Mount Vernon, WA in 2011; all samples were removed from open field plots after
final fruit harvest. A, discolored pith tissue and dry rot creating “ladder” effect; B, advanced stage where whole pith is rotted leaving hollow sections compared to C, a non-affected plant with green solid pith tissues and symptoms observed during field ratings included D, dark streaks forming acropetally along stem and petioles E, final leaf stages showing small chlorotic areas, and necrotic lesions extending from leaf margin to green midvein, F, adventitious roots breaking through stem tissues and forming along stem starting at mid-canopy, and G, tomato showing a ring of discoloration at blossom end. ……………………….. 85

7. Bacterial stem rot suspect on tomato cultivar Celebrity in an open field experimental plot laid with BioTelo mulch near Mount Vernon, WA in 2012. A, whole plant wilted within six days; B, hollow stem noted at site of wound caused during pruning; C, reddish-brown pith tissue from the upper canopy section; D, pitting around colonies of isolate Pbc.2012-5 on Hildebrand’s C medium (left) at 24 hours and (right) 13 days; E, oxidase test for isolates Pbc.2012-1 (left) as the negative control, Pbc.2012-3 (middle) and Pbc.2012-5 (left)………………………………………………………………………………………….. 86

8. Suspected early blight on leaves of tomato cultivar Celebrity grown with BioAgri (BioBag, Palm Harbor, FL) and black polyethylene (Berry Plastics Corporation, Evansville, IN) agricultural mulches in a study comparing high tunnel and open field production systems near Mount, Vernon WA in 2011…………………………….. 87

9. Disease symptoms on fruit harvested from experimental field trials evaluating five agricultural mulches in high tunnel and open field production systems near Mount Vernon, WA, 2011 and 2012. A, late blight affected tomato with bronze skin
discoloration localized to surface tissues; B, gray mold affected fruit, sporulation visible on the whole fruit and inner tomato tissues rotted; C, tomato with white mold, white mycelia at blossom end and sclerotia forming in rotted tissues.

10. Gray mold symptom severity scale on lettuce plants harvested from high tunnel and open field plots near Mount Vernon, WA in 2010, 2011 and 2012.
- A, Lesion on one leaf = 1% severity;
- B, some rot on three outer leaves and slight sporulation = 5% severity;
- C, one side of lettuce head affected, some rot and sporulation visible = 20% severity;
- D, most of crown infected, rot, wilt, chlorosis, and sporulation are visible = 50% severity;
- E, crown and head infected, rot and sporulation in crown and wilt, chlorosis and necrosis in head tissue = 70% severity;
- F, whole plant dead, rotted tissues have become dry = 100% severity.

11. Lettuce drop symptom severity scale on heads harvested from high tunnel and open field production systems near Mount Vernon, WA in 2010, 2011, and 2012. Severity of infection by S. sclerotiorum assigned
- A, 20% because infection progressed into inner tissues;
- B, 40% since most of the crown is infected, white mycelia are visible in crown tissues and rotted tissues have moved into portions of the head;
- C, 60% with the whole crown rotted and mycelium visible in head tissues with most of the plant wilted and rot starting to develop in inner leaves;
- D, 70% due to multiple areas rotted and visible sclerotia in crown tissues;
- E, 100% if whole plants had collapsed, leaves congealed, and crown rotted completely, or F, whole plant had dried up and black sclerotia were visible throughout tissues.

12. Symptoms of Verticillium wilt on harvested lettuce grown near Mount Vernon, WA in high tunnel and open field productions systems.
- A, patterns of either
v-notch or one-sided chlorosis on a few outer leaves; B, one-sided chlorosis and stunted growth (plant on right) compared to healthy plant (on left); C, one-sided vascular discoloration in crown tissues, as seen at harvest; and D, crown tissue with discolored vascular tissue placed in a moist chamber to isolate Verticillium spp.....

13. Tip burn observed on various lettuce cultivars grown near Mount Vernon, WA in high tunnel and open field plots during the 2010 through 2012 growing seasons. A, Tip burn on outer leaf from a marketable head; B, slight tip burn in lettuce head tissue, rendering the head unmarketable; C, severe tip burn extending down the leaf margin in outer leaves reducing head size; D, severe tip burn in Romaine cultivar Coastal Star. ................................................................. 145

14. Daily average air temperatures from 96 measurements per day in 15-minute intervals recorded by Hobo U-30 weather monitoring system in high tunnel (HT) versus open field plots (OF) at experimental field trials near Mount Vernon, WA in A, year 2010; B, year 2011; and C, year 2012. ................................................. 146

15. Comparisons between high tunnel (HT) and open field (OF) production systems for rainfall and leaf wetness during three consecutive lettuce growing seasons (April to June, 2010 to 2012). A, precipitation and B, leaf wetness is accumulated hours that leaf surfaces reached or exceeded a wetness threshold of conductivity above 50................................................................. 147

16. Photosynthetically active radiation (PAR) comparisons between high tunnel (HT) and open field (OF) plots at experimental trial near Mount Vernon, WA. Daily PAR is given as an average of 96 readings per day in 15-minute increments in A, year 2010; B, year 2011; and C, year 2012. ................................................. 148

xv
17. Percent relative humidity inside (HT) and outside (OF) high tunnels during spring lettuce growing seasons near Mount Vernon, WA as averaged across 96 recordings per day in 15-minute intervals in A, year 2010; B, year 2011; and C, year 2012.

18. Daily soil temperatures recorded at 5 cm depth within 23-cm raised beds covered with polyethylene mulch in an experimental trial near Mount Vernon, WA comparing high tunnel (HT) and open field (OF) plots with spring lettuce. Averages obtained from 96 recordings per day in 15 minute intervals in A, year 2010; B, year 2011; and C, year 2012.

19. Average daily wind speeds recorded in 15-minute intervals by a Hobo weather monitoring system located in a row within a high tunnel (HT) and open field (OF) plot near Mount Vernon, WA. Comparisons made between years in A, 2010; B, 2011; and C, 2012.

20. Vascular discoloration of greenhouse-inoculated lettuce plants. A, cultivar Merlot inoculated with strawberry isolate of *V. dahliae* to compare symptoms with B, Merlot inoculated with lettuce isolate of *V. tricorpus* and C, susceptible cultivar Salinas inoculated with the same *V. dahliae* and D, the same *V. tricorpus* isolates from 2010.
CHAPTER ONE

GENERAL LITERATURE REVIEW ON HIGH TUNNELS AND CROP MULCHES

Overview of High Tunnels

High tunnels are greenhouse-like structures that protect specialty crops from extreme weather, extend growing seasons, and increase crop quality and yield. High tunnels account for an estimated 582,902 ha (1,440,409 acres) worldwide in the production of vegetable and fruit crops (57), and are also used for ornamental plants. Photographs of high tunnels, raised beds, and lettuce crop rows are shown in Fig. 1. High tunnels differ from greenhouses in that the high tunnels utilize polyethylene covering in lieu of glass, are established in open fields atop native soil, are not heated, and generally do not use electricity. Tunnels are framed with various materials such as wood posts, plastic piping, or steel tubes. The frame, which is a series of hoops, is anchored to the ground, and is covered with a single or double layer of 0.10 to 0.15 mm clear, UV stabilized polyethylene film to allow optimal light penetration (57). High tunnels are similar to low tunnels except that high tunnel heights and widths are larger, and allow for full crop maturation and multi-row coverage (102). Although greenhouses provide many levels of precise environmental control including temperature, light, and relative humidity (103), these factors are costly and the structures are generally considered ‘permanent’, requiring a different permitting process than high tunnels which are ‘temporary’. In contrast, high tunnels provide some environmental control, and are cheaper to construct and operate due to low energy inputs for light, heat, and ventilation. High tunnels provide heat by trapping solar radiation, and are passively ventilated via open endwalls. Maximum ventilation is provided by rolling up sidewalls and opening endwalls during warm periods, and is a primary labor consideration in the operation
of a high tunnel. A water source is usually required for a high tunnel production system and drip irrigation with added fertilizer (referred to as fertigation) is a common practice (57).

High tunnels can be engineered to account for various climates, environmental conditions, available materials, and crop types (31; 57). The protective structures offer growers a competitive edge by increasing crop production while reducing the number of resources required to maintain plant vigor and control plant diseases and other problems. While the installation and management of high tunnels requires minimal skill (102), benefits of high tunnels can be negated if they are not properly installed and managed. The cost of materials and labor required to install, repair, ventilate, remove and relocate high tunnels is high relative to open field and low tunnel production systems, and several years of high tunnel crop production may be required to offset the costs. Material costs for high tunnel construction are estimated to be $1.50 per square foot compared to greenhouse construction costs of $8 to $30 per square foot (18). High tunnels have been shown to reduce production costs compared to greenhouse operations (31), and may reduce fungicide (107) and herbicide (101) applications. In recent studies in western Washington a single high tunnel structure (6 x 30 m) costs approximately $3,000 annually to operate, high tunnel management annual costs are estimated at $950, and depreciation costs are $430 per year (30).

**High tunnels and the environment.** Although high tunnel structures can be modified to adjust for temperature, humidity and wind, high tunnels are subject to prevailing conditions and structural adjustments may be needed to contend with extreme weather events. Growers in western Washington benefit from the accumulation of heat within high tunnels during spring and fall when open field temperatures are generally cool. Consequently, growers are able to transplant seedlings weeks or months earlier because frost injury is reduced (10; 30; 101; 103).
In addition, high tunnels extend growing seasons because retained heat delays plant senescence (10; 18). Solar energy accumulates as heat inside high tunnels due to restrictive air flow and the reflection of long wave radiation within the plastic cover. A study in New York showed that air temperatures inside high tunnels were higher than in open fields on sunny days: at least 10°C or higher in the center, and 7 to 8°C higher within 4.9 m of the open endwalls (104). A tomato production study conducted in Canada showed that high tunnel cropping systems increased overall air temperatures within the high tunnel by 2°C, and extended harvest by two weeks (102).

Timing and duration of ventilation is important in high tunnel cropping systems because temperatures can quickly rise and fall during sunny days and clear cool evenings. Plant vigor and fruit quality are reduced in heat-stressed tomato plants, and tomatoes will abort flowers if temperatures exceed 32°C or fall below 10°C thresholds (48; 102). Proper environmental control is especially difficult to maintain when planting more than one type of crop in a high tunnel due to competing growth requirements (102). For example, bell pepper plants placed alongside tomato in high tunnels had reduced vigor compared to plants growing in the open field due to the higher temperature that was maintained inside tunnels to benefit tomato (102).

Proper air flow throughout the growing season is also important to secure yield because high tunnels increase humidity, and thus, some plant diseases. Microclimates with high relative humidity can develop in high tunnels as plants mature, especially in crops with large canopies. Practices such as proper plant spacing and pruning may reduce some pockets of high humidity. Managing high tunnels to ensure appropriate growth during various stages of plant development is important to combat plant stress due to heat accumulation and to limit plant disease outbreaks due to high humidity. Also, crops which require insect pollination may not be properly pollinated if insect behavior is changed in the presence of a high tunnel structure. Ventilation or beehive
placement along the endwalls provides sufficient pollination for most outcrossing crops in high tunnels (31).

Careful consideration must be given prior to high tunnel installation in order to account for wind direction, light quality, and soil fertility. High tunnel placement and roof slope are important for the structure to sustain wind gusts, and improve solar radiation transmission (31). Some high tunnels are rated by the manufacturer for withstanding specific wind speeds. Placing sidewalls perpendicular to wind direction can create inward positive pressures along the sides of the structure and outward negative pressure along the roof (31); this placement allows for increased air movement when sidewalls are rolled up (10; 18). The positive pressure allows wind to blow into the structure along sidewalls and the negative pressures force wind up. In areas of high winds, the structures are more susceptible to damage. Since photosynthetically active radiation (400 to 700 nm) is reduced for crops located above and below 35°N and S latitudes, high tunnel roof slopes should be greater than 10 degrees to increase radiation transmission (31). Sloping the roof greater than 25 degrees may also reduce leaf wetness caused by dripping condensation within the high tunnel (31), and contributes indirectly to management of foliar diseases.

Fertilizers are usually applied to crops in the form of soluble salts which can build up in the soil over time, becoming toxic to plants through nutrient displacement or accumulation within plant tissues (37). Salt toxicity can cause stunting, chlorosis, leaf scorch, excision of plant tissues including root tips, and can alter reproductive development in some plants (2; 12; 82). Salt build-up from fertigation is a concern where high tunnel structures remain year-round in one location for consecutive years. A survey of 93 high tunnel production systems from farms located in Kansas, Missouri, Nebraska, and Iowa showed that salt accumulation was primarily in
the upper 5 cm surface compared to 0-15 cm within open fields based on electrical conductivity tests (50); however, salt accumulation was not dependent on length of time under the high tunnel. High tunnel relocation and soil flushing were recommended to avoid salt toxicity. In temperate regions such as western Washington, three-season high tunnel structures are removed following fall harvest and thus rain and melted snow are allowed to penetrate and flush the soil.

**High tunnels and plant diseases.** High tunnels modify many environmental parameters including leaf wetness, soil moisture, and air and soil temperature, providing opportunities to also modify the occurrence and spread of plant diseases (10; 103; 105). In addition, high tunnels offer some protection from wounding caused by excessive winds and hail, thereby shielding plants from opportunistic pathogens. The use of raised beds and canopy shading are a few examples of cultural techniques used to alter the environment and microclimate within high tunnel systems. These practices improve soil drainage and reduce plant stress, and can sometimes also increase crop yield by reducing plant disease (91). Resistant cultivars, proper irrigation, and plant pruning and spacing can also reduce plant diseases in high tunnel systems (10).

Well-ventilated high tunnels can inhibit or reduce spore production as a result of decreased leaf wetness. Damping-off and root rots in moist soils caused by *Pythium* spp. Pringish and *Phytophthora* spp. de Bary are examples of diseases which can be partially mitigated by the higher temperatures achieved inside high tunnels (10). Several studies have shown a reduction of late blight, caused by *P. infestans* (Mont.) de Bary, in tomato production by using protective structures (53; 95). Tumwine *et al.* (95) demonstrated that high tunnel structures and removal of infected plant tissues (a form of sanitation) in Uganda reduced the incidence of late blight by approximately 17% compared to a fungicide treatment. In 2010, six early-maturing tomato
cultivars were grown in open fields and high tunnels in western Washington and all cultivars were susceptible to late blight, but disease incidence and severity was lower inside the high tunnels than in open fields (39).

Although high tunnels provide protection from pathogens such as *P. infestans*, other pathogens can be favored by the environment within the structures. Powdery mildew, gray mold and leaf mold are examples of plant diseases that can sometimes be favored by high tunnel cropping systems because they thrive in warm or humid conditions which can occur in high tunnels with poor ventilation (10; 74; 105). Powdery mildew occurs on dry foliage but requires high relative humidity for spore germination (24; 34), and gray mold develops in pockets of high relative humidity resulting from poor air movement in dense canopies or in moisture from fog or condensation (108). If proper ventilation is achieved, gray mold can be reduced in high tunnels compared to open fields. For example, strawberries grown under high tunnels in Florida without fungicides had an 89% reduction in gray mold incidence compared to open fields that were on a seven-day fungicide application cycle (107). The authors attributed the reduction in disease to the 60% reduction of leaf wetness inside the tunnel compared to the open field. These high tunnels offered significant protection against *Botrytis cinerea* Pers. ex Fr. (teleomorph *Botryotinia fuckeliana* Whetzel), and even when a seven-day fungicide application cycle was applied inside the high tunnels, gray mold incidence only diminished further by 2%. Another study also revealed limited gray mold on strawberry fruit grown in high tunnels in western Washington (40). The high tunnels limited gray mold due to lower soil moisture, higher soil temperatures, and by eliminating 654 hours of accumulated leaf wetness.

Higher soil temperatures inside high tunnels can also favor pathogen growth and infection on susceptible hosts, as seen with strawberries infected with *Verticillium dahliae* Kleb.
in western Washington (40). Soil temperatures were 0.95 to 2.44°C higher and disease incidence was 8.3% greater in high tunnels than in open fields in that study. The average maximum soil temperature (5 cm depth) inside high tunnels only reached 18.3°C while the average open field soil temperature was 14.2°C. *V. dahliae* propagules, called microsclerotia, are dormant in soil but germinate in the presence of plant exudates. Microsclerotia can be induced into mycostasis by other soil microorganisms (28; 65), and therefore, soil temperature and/or moisture conditions that affect antagonists of the microsclerotia of *Verticillium* can also alter microbial-induced sclerotial dormancy. Pegg and Brady (73) provide a review of Verticillium wilt infections based on host and temperature. Other studies have shown that *V. dahliae* is capable of causing disease on susceptible hosts grown at 30°C (41).

**Overview of Mulches**

Plant debris such as straw, hay, and clippings is the oldest, most readily available, and least expensive mulch used in agriculture. Mulch is laid on top of the soil and provides several benefits to production systems including weed control, moisture retention, and temperature modification. Many plastic mulches block sunlight transmission thereby reducing herbicide use, increasing plant vigor by suppressing weed germination and competition, and providing more efficient irrigation through limited water loss via evaporation (55-57). While these added benefits are useful in both organic and conventional cropping systems, the weed control attributes of plastic mulch are very important in organic agriculture as there are no effective herbicides available for use. Dark-colored plastic mulches are typically laid on top of raised beds prior to planting, and the crop is sown or transplanted through holes cut in the plastic. Plastic mulches are commonly used with high tunnel systems.
Linear low density polyethylene films are the most common types of plastic mulch due to bulk production, which helps to limit costs. This mulch can be applied to raised beds with special machinery, and has desirable physical properties such as flexibility and durability (55). Black plastic mulch increases soil temperatures as deep as 10 cm and by as much as 1.7°C because the mulch absorbs most UV, visible, and infrared wavelengths (55; 56). However, energy transfer to the soil in the form of thermal radiation decreases as the contact area between the mulch and the soil increases (55). Increased soil temperatures can enable earlier planting and reduce crop loss by limiting transplant shock and promoting root development. However, loosely fitted mulch films can also cause seedling injury due to wind lift.

Mulch color may affect plant growth and disease due to reflected energy into the canopy and changes in temperature in the root zone (21; 22; 42). For example, lettuce head weight and diameter were significantly higher when surrounding soil was covered with an infrared transmitting green mulch rather than white-on-black polyethylene; but tip burn, caused by calcium deficiency, was not affected by various colored mulch films (42). Tomato fruit production was not significantly different with black or white mulch (21). However, Díaz-Pérez et al. (22) showed that tomato spot wilt virus symptoms increased when black mulch was used compared to gray or white mulch after a 15-to-36-day mulch incubation period prior to virus inoculation, and the black mulch reduced fruit yield. When plants were inoculated without a mulch incubation period, no disease difference was found among the mulch treatments, indicating that the warmer soil temperatures produced by black mulch compared to bare soil likely increased systemic movement of the virus within the plant.

Paper mulches are similar to polyethylene mulches in providing a uniform groundcover, and are also used in agriculture and may warm the soil effectively. However, one study suggests
that paper becomes bleached during the growing season and this outcome reduces paper mulch soil warming properties (83). Another study showed that WeedGuardPlus, a commercially-available cellulose mulch, had significantly greater number of rips, holes, and tears in open fields compared to BioAgri and BioTelo, two commercially-available biodegradable mulches, but degradation of WeedGuardPlus was not significantly different from these other mulches inside high tunnels (67).

**Biodegradable mulches.** Plastics used for high tunnels, mulches, and irrigation tape are expensive to remove, and may not be recyclable due to contamination by soil and agricultural chemicals (56). Polyethylene is resistant to biodegradation because it is hydrophobic and has a high molecular weight, so that hydrolysis of the carbon backbone and the potential for microbial assimilation is limited. Polyethylene is also resistant to photolysis and abiotic degradation due to manufacturing processes that add anti-oxidants and stabilizers (109). The development of plastic mulches that photodegrade or biodegrade is of current interest to growers and industry producers. Such materials could eliminate the cost of removal and disposal, and could potentially be incorporated into the soil to provide a carbon source for soil macro- and microfauna.

Bio-plastics are generally separated into two groups: bio-based plastics and biodegradable plastics. Bio-based plastics are produced from biological sources such as plant starch (44), whereas biodegradable plastics can be plant or petroleum-based but can be degraded by microorganisms (92). Bio-based plastics can be blended with petroleum-based plastics and end products can vary in level of degradation (109). Partial biodegradation of plastic films is not desirable because the fragments can adsorb toxic substances such as pesticides from the soil or from the water (58; 71). These toxin-coated plastic fragments (from visible to microscopic in size) may enter the soil or aquatic food webs, where they are hazardous to animal and human
health (70). In addition, some plastic blends of have undesired properties such as brittleness (109). The incorporation of biopolymers with pro-oxidants like iron dithiocarbamate and various metal stearates (4; 14; 78) is thought to be a possible means of providing a fully biodegradable plastic mulch (109). Additives such as dyes, thermal stabilizers, and anti- or pro-oxidants can also be incorporated to achieve desired properties such as flexibility and high melting point. Ideally, biodegradable plastic mulches used in agriculture should be durable, such that they provide sufficient coverage during the growing season to avoid weed competition and maintain efficient watering regimes. The plastics should also completely degrade into water and carbon dioxide and tested according to ASTM International standards (70; 71). If biodegradable mulches were incorporated into the soil post-harvest, the plastics would need to completely break down within three to six months to allow for subsequent season cropping cycles. Several problems arise when incorporating biodegradable mulch into a soil system. Foremost, mulches would be incorporated into the soil at the end of the growing season, when soil conditions are cool or soils may become saturated; these conditions are not conducive to microbial degradation. Other complications can arise because plastics are hydrophobic; this property deters microbial colonization and subsequent enzymatic degradation. Additionally, the chemistry of available materials used in plastic production to provide desired qualities may not simultaneously allow enzymatic attack due to their structure. Several reviews on biodegradable and synthetic plastics explain their benefits, limitations, and biodegradation (27; 69; 92; 99; 109).

Poly(lactic) acid (PLA) is one type of bio-based plastic manufactured from starch. In this process, starch is fermented to create lactic acid and subsequent chemical reactions result in the synthesis of L- and D-PLA isomers (44). PLA is a linear aliphatic polyester, but has weak biodegradation ability because microorganisms that degrade PLA are not abundant or widely
distributed, and the D-PLA isomer does not break down easily via known proteases (92).

However, PLA is derived from renewable resources and has high tensile strength, 50 to 70 MPa (43). Polyhydroxyalkanoates (PHAs) and polyhydroxybutyrate (PHB) are polyesters produced by several bacterial genera. These polymers are stored inside the cell as energy reserves (46), and can account for 30 to 80% of cellular dry weight when the bacteria are stressed by specific nutrient deficiencies (3). PHAs and PHB can be produced in large quantities via fermentation processes with substrates such as glucose (3). Poly(butylene adipate-co-terephthalate) (PBAT) is a biodegradable aliphatic-aromatic copolyester (43) that undergoes photodegradation through main chain scission and crosslinking (45).

Soil microorganisms secrete various enzymes which break down polyester chains via esterases, lipases and proteases. PLA is degraded by Fusarium moniliforme, Penicillium roqueforti, Pseudomonas putida, and Amycolatopsis sp. (44; 93; 94). Paenibacillus amylyticus secretes protease and esterases which break down PLA and PBAT; however, these degraded carbon sources are not utilized by the bacterium (90). Failure of microorganisms to accumulate the degraded substrates or excrete extracellular depolymerases can be attributed to inadequate temperature, pH, hydrostatic pressure, and substrate specific binding sites (3; 46). PHAs are degraded by various microorganisms: Terrabacter tumescens strains MB-14, MB-15; Duganella zooglooides strains MB-9, MB-13, MB-17, and MC-9; Pseudomonas lemoignei strains MB-12 and MC-2; Matsuebacter chitosanotabidus strains MB-7 and YB-11; Variovorax paradoxus strains MB-16, WFF52; various Type 0803 filamentous bacteria; and, one iron-oxidizing lithotroph in the Xanthomonas group (89). Biodegradation of mulches within agriculture systems depends greatly on environmental factors and the presence of microorganisms capable of degrading and utilizing carbon from biodegradable films.
Mulches that are labeled as fully biodegradable exist in the marketplace (70; 71). For example, there are two starch-derived black films containing the feedstock Mater-Bi. One experimental Mater-Bi mulch (Novamont S.p.A.) made for warmer climates was used on tomato crops in Spain, where field conditions were capable of initiating degradation processes. Tomato yields in plants mulched with Mater-Bi also were the same as plants mulched with polyethylene mulch, but were higher than yields from plants mulched with paper and no mulch treatments (61).

**Mulches and plant diseases.** Crop yield is the primary focus of many studies comparing plastic mulches. There is limited information about mulch effects on plant diseases. As mentioned above, the heating effects of black mulch reduced the number of days for symptom development of selected viruses compared to light colored mulches (22). In another study, silver mulch eliminated tomato spot wilt virus (TSWV) and cucumber mosaic virus infection, and white, green, and black colored mulches reduced the number of TSWV affected plants compared to a bare ground control (60). The same study showed that severity of corky rot (*Pyrenochaeta lycopersici* R. Schneider & Gerlach) of tomato was reduced with silver mulch compared to other colored plastic mulches, but corky rot severity was similar to the non-mulch control (60). In this study soil temperature affected disease incidence as silver mulch and bare ground was 5 to 8°C cooler than white, green and black colored mulches. Incidence and severity of Rhizoctonia fruit rot of cucumber can be significantly reduced, up to 75%, when photodegradable black plastic mulch and plowing are used in an integrated pest management system (59). In this study mulch was a barrier that inhibited contact between fruit and the soilborne pathogen.
Common Diseases on Tomato in Western Washington

Tomato (Solanum lycopersicum, formerly Lycopersicon esculentum Mill) is mostly grown in western Washington for fresh market sales (6). Several pathogens affect Solanaceous crops in Washington, including: *Alternaria tomatophilai* Sorauer (formerly *A. solani*, early blight); *Botrytis cinerea* Pers. ex Fr. (teleomorph *Botryotinia fuckeliana* Whetzel; gray mold); *Fulvia fulva* Cooke (syn. *Cladosporium fulvum*; leaf mold); *Fusarium oxysporum* f. sp. *lycopersici* (Sacc) Snyder & Hans. (vascular wilt); *Phytophthora infestans* (Mont.) de Bary (late blight); *Pythium* spp. Pringish (damping off); *Rhizoctonia solani* Kühn (damping off); *Sclerotinia sclerotiorum* (Lib.) de Bary (white mold); and, *Verticillium dahliae* Kleb. and *V. albo-atrum* Reinke et Berth (Verticillium wilt) (68). Migratory beet leafhoppers can infect tomatoes with the beet curly-top virus (23; 68). The most problematic pathogens of tomato production in the U.S. are *P. infestans* and *B. cinerea* (32; 80). An emerging disease is tomato pith necrosis, caused by *Pseudomonas corrugata* Scarlett (54).

Late blight. Late blight is a common problem for tomato production in temperate regions and is considered by many to be the most important disease of tomato worldwide. Late blight epidemics occurred in the U.S. during the 1990s and affected tomato and potato (both solanaceous crops). These serious epidemics were caused by new strains of *P. infestans*, US-7, US-11, and US-17, all resistant to the systemic fungicide metalaxyl (29). A subsequent late blight epidemic occurred on tomato early in the 2009 growing season, mostly in the eastern U.S., after diseased seedlings were distributed from contaminated nurseries and the growing season weather conditions proved highly favorable. The new strain, US-22, quickly spread (63; 110). *P. infestans* is a heterothallic Oomycete which survives in soil as oospores for months to years or in plant material such as tomato stems and potato tubers as mycelia for days to weeks. Oospores
resist desiccation due to thick cell walls that are composed primarily of cellulose and β-D-glucans. Oospores have been identified in some places of the world, but have not been detected in the U.S. Since *P. infestans* is an obligate necrotroph, crop rotation is an effective control measure to eliminate soilborne but not airborne inoculum.

Symptoms of late blight appear shortly after periods of cool, moist weather. Symptoms start as water-soaked areas on foliage, stems, or fruit, where the pathogen degrades the cell walls of the host. Water-soaked areas become sunken, turning dark green, and then develop into dark lesions which rapidly become white as sporulation ensues. Signs (sporangiophores and sporangia) of the pathogen are visible within three days after prolonged moist conditions. Sporulation requires high humidity (91 to 100%), with optimum formation occurring with temperatures between 18 and 22°C (19). Sporulation primarily occurs on the abaxial surface of foliage, and is often most easily visible in the early morning. Necrotic lesions enlarge and become brittle. Diseased fruits are firm but skin becomes discolored, with a characteristic bronze color. Lesions may contain millions of sporangia, each containing hundreds of spores. The sporangia disperse in the wind and land on adjacent plants.

*P. infestans* spreads rapidly and is difficult to control due to its polycyclic nature and the rapid, abundant production of spores, which also leads to new strains with heightened fitness, virulence, and/or fungicide resistance. Strain US-7 is an example of a new strain with A2 mating type and metalaxyl resistance, which makes sexual recombination possible with an A1 mating type such as US-11 or US-17 (110). There have been no reports of sexual recombination between mating types in the U.S., but the presence of both mating types may increase the chance of genetic variation leading to additional virulent strains in the future (63). Late blight is controlled primarily through protective fungicide applications, and forecasting models are available that
correlate time of spray with favorable environmental conditions such as rain, humidity and temperature (33). Sanitation and roguing practices that eliminate infected plant tissues and inoculum, are both important management techniques, but are costly to employ due to time requirements for monitoring, removal, and disposal of infected plant material. Moreover, these practices are effective only on a temporary basis. Removal of Solanaceous weeds like nightshade is also important because weeds can be hosts to P. infestans. Breeding for resistance is very important, but currently there are no tomato cultivars available with a broad range of resistance (64). Consumer preferences and climatic constraints may also limit the choices of cultivars grown within a region. In western Washington, where the growing season is limited due to low accumulation of growing degree days at 10°C base temperature, cultivars with early-to-midseason maturity must be grown. Six tomato cultivars were evaluated in this region for late blight resistance in 2010, and all cultivars were susceptible (39). Area under disease progress curve values were not significantly different among cultivars, although percent blighted fruit was highest in Celebrity and lowest in Stupice.

Gray mold. Gray mold is another common tomato pathogen which is difficult to control because the causal agent, B. cinerea, is capable of infecting many crops (>200 hosts), has saprophytic capabilities, and strains with fungicide resistance have been identified in Washington (15; 47; 111). B. cinerea is capable of infecting both young and mature plant tissues (111). The pathogen can cause damping-off of young seedlings and attack mature tissues, causing stem cankers and foliar, flower and fruit blights. In addition, the pathogen produces survival structures called sclerotia, which allow the pathogen to persist in fields and greenhouses as primary inocula. These sclerotia germinate and produce conidia asexually. The spores are capable of germinating and infecting plant tissues within five to eight hours whenever plant tissues have a
thin water film from dew, fog or rain, and air temperatures are between 15 and 24°C (80; 111). Spores land on flowers or wounds, and remain viable for hours to weeks. Spore viability is highly dependent on sunlight exposure, and viability decreases as exposure to light increases. Spore germination can be stimulated by pollen, and pollen grains provide a nutrient base that may increase virulence (111).

Gray mold symptoms appear in early spring and late fall, or whenever the environment is cool and wet. Small brown water-soaked lesions may be visible within three days of infection. Masses of spores are spread by wind or splashing water (111). Tomato fruits can become infected when spores of the fungus colonize the flower petals (97) or when fruit is compromised from blossom end rot, a calcium deficiency which weakens cell walls. Infected fruits rot and have an unusual appearance because the fruit skin remains intact and creates a clear paper-like sack around the pericarp. Tomato fruit yields can be greatly reduced when infections occur on the stem; these often occur at the site of wounds caused by pruning, staking, and trellising. Stem cankers block the flow of water and nutrients, stunting plant growth; when severe, stem girdling may cause death (97). After colonizing and decaying the host, the fungus produces dry, brown conidia on conidiophores. Multiple sporulation events may happen on the same plant and severe epidemics can occur if environmental conditions remain favorable.

Controlling gray mold involves modifying environmental conditions so they are not favorable for pathogen growth, or adjusting cultural practices to increase temperature and reduce moisture and humidity. Plant infection and spore production are both promoted by cool and humid environments, conditions that are often common later in the tomato growing season and can contribute to reduced fruit yields (11). Growth and sporulation of *Botrytis* spp. is inhibited at temperatures above 28°C (111), and although it is unlikely that temperatures remain this high...
during the evening in western Washington within high tunnels, the heat which is trapped during the day may inhibit growth, slowing disease progression. A study in Florida showed that high tunnel use in strawberry production reduced Botrytis fruit rot by 88 to 94% compared to the open field due to reduction in leaf wetness during rains (107). Pruning, adequate plant spacing, and other ventilation practices that increase air flow, will help to lower humidity below 80%, which is the ultimate goal for disease management. Currently, there are no gray mold-resistant cultivars available, in part because B. cinerea is capable of detoxifying alpha tomatine, a saponin of tomato. Botrytis is thought to have multiple saponin-specific enzymes (77). Some tomato cultivars are more susceptible to gray mold than others, but disease incidence varies depending on environmental conditions (11). Sanitation can help reduce secondary spread, which occurs when infected plant tissues provide inoculum to infect other plant tissues. Tools surface-disinfested with 70% ethanol should be used to help control spread, and severely infected plants should be removed carefully because disturbed plants easily shed spores.

Tomato pith necrosis. P. corrugata, a soilborne bacterium with a wide distribution and metabolic capabilities, is the causal agent of tomato pith necrosis. P. corrugata is an opportunistic pathogen which has been identified in bulk field and rhizosphere soils. Due to its wide array of metabolic capabilities, P. corrugata is also studied as a biocontrol agent against take-all of wheat and in soil remediation (9; 79; 100). Symptoms of tomato pith necrosis develop on lower leaves which first turn chlorotic and then necrotic. Dark lesions develop on the stem and petioles and progress acropetally. Pith discoloration is visible in advancing diseased tissues, and often takes-on a ‘corrugated’ appearance before eventually becoming hollow and collapsing. The plant wilts as the disease progresses and some cultivars produce abundant adventitious roots near or above the soil line. Disease requires cool evening temperatures, high humidity, and
wounded or soft tissue. Excess nitrogen is thought to cause soft tissue due to rapid plant growth, and may contribute to disease outbreaks (16; 62; 84). Early planting of tomato may lead to greater disease susceptibility. Protecting plants from cool night temperatures and modifying relative humidity helps to limit the disease in tomato cropping systems. Ensuring that appropriate nitrogen levels are supplied during transplanting and allowing for proper ventilation to reduce humidity are crucial in controlling the disease. According to various extension publications, tomato pith necrosis in the U.S. is seen in tomato production systems that use mulch (16; 62; 84). The disease was observed in open field tomato production where several agricultural mulches and bare ground systems were used near Mount Vernon, WA in 2011 (see Chapter 2). Virulence differences as defined by extent of pith necrosis advancement from inoculation sites have been noted among isolates of the pathogen tested on tomato and eggplant.

**Common Diseases on Lettuce in Western Washington**

Lettuce (*Lactuca sativa* L.) is a cool weather crop and is susceptible to pathogens in western Washington that grow actively under cool, wet conditions. *Bremia lactucae* Regel (downy mildew), *B. cinerea* (gray mold), *Pythium ultimum* Trow and *R. solani* (damping off), and *S. sclerotiorum* (lettuce drop) are common lettuce pathogens in Washington (68). Of these, *B. cinerea* and *S. sclerotiorum* are common problems in commercial production of lettuce within the U.S. (51; 72; 87). In addition, Verticillium wilt caused by *V. dahliae* or *V. tricorpis* I. have been reported as primary pathogens on lettuce throughout the U.S. and Japan (76; 96).

Gray mold and lettuce drop. *B. cinerea* is primarily saprophytic, but also is an opportunistic pathogen that can infect young or mature injured or senescing tissues, whereas *S. sclerotiorum* is an obligate necrotroph which infects senescing tissues. Both pathogens have a
wide host range and produce resting structures called sclerotia. *B. cinerea* infects over 200 plant species (111) and *S. sclerotiorum* infects over 400 plant species (13; 75). *B. cinerea* secretes two phytotoxins, botrydial and botcinic acid, which contribute to virulence, and orthologs have been found in *S. sclerotiorium* (20). *S. sclerotiorum* secretes oxalic acid, which changes the pH within the host and leads to production of polygalacturonases and enzymatic degradation of cell walls, inhibition of plant defense responses, and apoptosis-like cell death of the host (5; 20; 106).

*B. cinerea* is considered a weak pathogen on lettuce and requires vulnerable plants such as those that are young, wounded, or have senescing tissues; actively growing tissues are rarely penetrated (8). High humidity (>80%) is required for the pathogen to penetrate plant tissues but infection can occur over a wide range of temperatures. Infections at temperatures of 0 to 10°C have been reported, but optimum infection occurs at 18 to 23°C and rarely at 25 to 28°C (8). Once the plant is infected, colonization can occur between 0°C and 35°C (8). UV light stimulates production of asexual spores, and these conidia are often the infectious propagules that land on plant tissues, germinate, and infect the host. Brown lesions first appear on infected areas which expand, leading to rot, that turns leaf or crown tissues into slime, and dry masses of gray, powdery conidia form in the center of the lesions. In crown rot, *Botrytis* infection can be rapid. Outer leaves may become chlorotic as vascular tissues become rotted, and often lettuce heads quickly wilt while retaining green pigment. Brown, soft rotting midveins appear in lower leaves and may extend to the leaf tip within hours to days. Rotted crown tissues have a characteristic orange-brown color bordering the gray mass of conidia. Sclerotia are melanized, plano-convexoid shaped structures, approximately 3 to 5 mm in diameter, that may be visible in advanced disease stages, can serve as overwintering structures, and are capable of surviving
temperatures from 4 to 54°C (8). Sclerotia can also germinate and produce conidia under cool, moist conditions. However, not all Botrytis isolates produce sclerotia.

Overcast skies, fog, and rains are all environmental factors that increase gray mold epidemics. Frost, sun scorch, tip burn, mechanical injuries, or senescing tissues are infection courts for B. cinerea. Applying calcium helps reduce tip burn, and therefore, may also reduce gray mold. Foliar calcium applications may be required if overcast and cool conditions prevail because calcium moves through the plant via transpiration which is reduced under these conditions (85). Plant spacing that accommodates canopy closure without the need for thinning is an important cultural disease control method as it provides improved ventilation, which reduces conidial production and survivability. Another cultural disease control practice is irrigation: surface irrigation eliminates leaf moisture and large pockets of humidity as compared to overhead irrigation. Steaming soil at 71°C for one hour or 81°C for thirty minutes is effective in reducing populations of sclerotia in soil, but this practice is only commonly carried out in greenhouse operations (8). Several studies suggest using treated seeds and transplanting young seedlings because immuno-labelled mycelium of B. cinerea has been visualized in nonsymptomatic lettuce plants, providing evidence that the pathogen is vertically transmitted via seeds (86). Also, lettuce is more susceptible to crown rot when overgrown seedlings are transplanted because older plant tissues are more prone to damage and older seedlings often have some senescing leaves (51).

S. sclerotiorum causes lettuce drop, a disease that results in plant collapse due to girdling and rot at the lettuce crown. Sclerotia are produced in dying plant tissues and can survive 4 to 10 years in soil (81). Sclerotia undergo myceliogenic or carpogenic germination, respectively, forming either mycelia or sexual reproductive structures. Sexual structures are apothecia that
function to release ascospores. Sclerotia germinate when dormancy is broken and germination occurs when high soil moisture content washes away water-soluble dormancy factors. Germination can occur between 10 to 28°C and higher numbers of apothecia are produced when small temperature fluctuations occur with constant soil moisture near maximum water holding capacity (-0.09 to -0.1 MPa) compared to low saturation levels (-0.03 to -0.04 MPa) (66). Carpogenic germination leads to the production of ascocarps (apothecia) from which ascospores develop and become windborne. Sclerotia can produce apothecia multiple times over a life cycle and multiple apothecia may be produced at the same time from a single sclerotium. Apothecia formation contribute to fungal survival, but apothecia have only been found when the sclerotia are located within the top 2 cm of soil due to stipe length restrictions. Ascospores are continuously released regardless of light cycles, but release is affected by relative humidity. Ascospore release decreases after 48 hours when relative humidity is between 65 to 75% compared to 90 to 95%, and survival is affected by temperature as viability is reduced at temperatures > 25°C (17). Survival rates are high when relative humidity is between 80 to 93%, and 50% spore survival occurs when air temperature is 25°C after two weeks (17). Ascospores store little energy and the spores require senescing tissues to germinate and infect healthy plant tissues (106). Infected flower blossoms and tissues fall and land in the soil, leaf axils, or other plant parts. Infection by S. sclerotiorum on detached bean leaves was shown to occur in a moist chamber between 10 to 25°C; ascospores on water or potato dextrose agar media infected detached lettuce leaves at 15°C within one week of exposure when kept in a moist chamber (1; 72). Myceliogenic germination of the sclerotia can also result in infection through direct penetration of healthy tissue (38). However, infection by mycelia is thought to occur rarely as it requires sclerotia to be in close proximity (approximately 2 mm) to plant stems.
Symptoms of lettuce drop start as water-soaked lesions on the leaves, followed by rotting and abundant white mycelial growth if wet conditions prevail. When the pathogen infects crown tissues, water and nutrients are blocked and the plant becomes wilted and chlorotic. Eventually the whole crown rots and the plant collapses. Long, flat, irregular shaped, dark sclerotia bodies can be seen embedded in dying plant tissues.

Due to a wide host range and long survival times in soil, lettuce drop is difficult to control through crop rotation. Other factors such as population variability and inoculum source also make this pathogen challenging. *S. sclerotiorum* undergoes sexual reproduction in nature and some populations contain high genetic variability. For control, protective fungicide sprays are applied just prior to ascospore release. However, ascospore production can persist for extended periods of time if environmental conditions are favorable. Because ascospores can travel long distances in air currents, up to 3 to 4 km, ascospores from neighboring areas may also cause infections as was demonstrated by a study on efficacy of the biocontrol agent *Coniothyrium minitans* to control white mold on potato in the Columbia Basin of Washington (35). Disease management strategies include reducing humidity by plant spacing, and increasing temperature via row covers and high tunnels. As mentioned previously, high temperatures reduce sclerotia germination and both clear and black plastic mulches increase soil temperatures in both high tunnels and open fields. To date, plastic mulch has not been studied as a cultural practice to block ascospore dispersal or restrict the air currents that are required to transport ascospores.

**Verticillium wilt.** *Verticillium spp.* can infect lettuce but disease development is dependent on pathogenic races, susceptible cultivars, soilborne inoculum, and favorable environmental conditions. *V. dahliae* is a serious pest on lettuce and strawberry in areas of California. *V. dahliae* is highly pathogenic on lettuce and two races have been identified, race 1
and race 2 for this crop (98). There are no resistant lettuce cultivars for *V. dahliae* race 2 (36). U.S. isolates of *V. tricorpus* are considered mildly pathogenic on lettuce (76). A virulent strain of *V. tricorpus* has recently been reported on lettuce in Japan, and this strain is genetically distinct from California isolates. However, isolates of *V. tricorpus* from Japan are only mildly virulent on potato or are nonpathogenic on many other vegetable crops (26; 96). *V. tricorpus* is primarily saprophytic with a narrow host range but it can co-colonize lettuce root tissues with *V. dahliae* (76). *V. tricorpus* appears to reduce disease severity when co-inoculated with *V. dahliae* as compared to lettuce plants inoculated with *V. dahliae* alone (76).

Symptoms of Verticillium wilt develop after lettuce head formation. In greenhouse trials, foliar symptoms developed around 10 weeks post inoculation (7). Epidemics can cause large losses for growers because infection only becomes evident after expensive inputs such as fertilizers and irrigation have been applied. Symptoms of one-sided chlorosis and wilt develop on lower leaves, flagging continues as disease progresses, v-notched chlorosis lesions develop at leaf margins, and leaf necrosis can follow. As disease progresses and/or temperatures increase, disease symptoms worsen and plants can rapidly wilt and collapse. Green-black discoloration can be noted in the vascular tissues of lettuce crowns and roots, and is the result of successful colonization and subsequent interruption of water transport due to conidial production and stimulation of tylose formation. Although the green-black vascular discoloration noted in lettuce crown and root tissues infected with *V. dahliae* is well documented (88), specific patterns of discoloration have not been recognized in infections caused by *V. tricorpus*.

Lettuce crops require high *V. dahliae* soil inoculum density, 100 or more microsclerotia per gram of soil, for disease development compared to other crops like strawberry and cauliflower where inoculum density thresholds are as low as 2 and 10 microsclerotia per gram of
soil, respectively (7). Root exudates stimulate microsclerotia to germinate, but the microsclerotia of *V. dahliae* must be within 2 mm of host tissue, perhaps due to limited energy resources or diffusion limitations of signaling compounds when dormancy is caused by microbial suppression. Hyphae of the fungus penetrate host root tips near the zone of elongation, at root hairs, or near ruptures in lateral roots (73). Hyphae can penetrate the cortex without forming appresoria, and grow through cells forming callose deposits. Pathogenic strains are capable of colonizing vascular tissue whereas nonpathogenic strains do not advance beyond cortical cells. For example, *V. tricorpus* is pathogenic on tomato but not cotton, but root penetration has been observed for both plants (73). High temperatures accelerate the symptoms of wilting since the pathogen occupies vascular tissues which become plugged with fungal mycelium, spores, and tylose and gum deposits from plant defense responses. Microsclerotia that develop in senescing tissues and roots are incorporated into soil where they remain viable for months to years.

Soil populations of *Verticillium* are difficult to control for several reasons: (i) microsclerotia can persist in the soil for long periods of time—reports of viability range from months to over 10 years; (ii) *V. dahliae* has a wide host range, infecting over 200 crop species which renders crop rotation ineffective; (iii) *V. tricorpus* has saprophytic abilities, and does not require a host in order to survive, but may thrive when a host is incorporated into the rotation scheme; (iv) *V. tricorpus* has also been isolated from non-symptomatic weed species and therefore green bridges for both of these two *Verticillium* spp. may exist; (v) *Verticillium* can be seedborne, which may be a means of introducing new races with increased virulence into an area; (vi) there are no resistant lettuce cultivars currently available for *V. dahliae* race 2; (vii) soil amendments are not always effective at reducing inoculum potential and they produce varied results from location to location; (viii) soil fumigation can be effective, but not always for the
long term, and there are drawbacks to fumigation due to pathogen rebound via loss of antagonists not to mention that fumigants are heavily regulated and costly; and (ix) soil solarization can be effective in reducing inoculum, but is not as effective at high latitudes where sun light intensity is reduced—microsclerotia must be heated to 50°C for at least 30 minutes for effective control, according to some reports (73).

For Verticillium wilt, the presence of certain vegetative compatibility groups (VCGs) within Verticillium species can potentially affect disease occurrence. Crops may be susceptible to many VCGs as reviewed by Klosterman et al. (49). For example, V. dahliae VCG 4B has been reported pathogenic on lettuce. Additional studies are needed to confirm that other VC groupings are not pathogenic on lettuce (25). VC groupings for V. tricorpus have not yet been reported on lettuce, but interestingly Korolev and Katan (52) report that two isolates of V. tricorpus from Israel formed a complementary heterokaryon with a single V. dahliae isolate from VCG 2B.

**Experimental High Tunnel and Mulch Trials**

Given the short duration of optimal growing conditions in western Washington, growers in the region could benefit from production systems that extend cropping seasons. Since there is interest and demand for locally grown organic crops, high tunnels could also potentially increase fresh market vegetable quantity and quality, especially during periods of cool temperatures or heavy rains.

This study evaluated specialty crops, tomato and lettuce, to determine if high tunnel production systems in western Washington influence disease outbreaks, particularly foliar diseases, under primarily organic growing conditions. Further, since plastic mulch is commonly used in organic high tunnel production as a form of weed control in lieu of herbicide
applications, and many soilborne pathogens are affected by soil moisture and temperature, the effects of mulch on plant disease was also investigated.

The high tunnels used in this study were part of a USDA-AFRI SCRI-SREP project (No. 2009-51181-0587) during the years 2010 to 2012. The open-ended structures (‘Solo’ model, Haygrove LTD, UK) were 37 m long x 8 m wide x 3 m high, and allowed tractor access in the center. High tunnels were erected at Washington State University Mount Vernon NWREC for three consecutive cropping cycles, begining in mid-April each year, prior to transplanting lettuce and tomato seedlings, and removed mid-to-late October, after final tomato harvest. The field site had been managed organically with small grain and legume rotations for four years prior to this study. Soil was amended with organic fertilizer Par4 9-3-7 (North Pacific Ag Products, Portland, OR) at 90 kg N·ha⁻¹ applied to bed centers prior to forming beds. Beds were shaped (90 cm wide x 37 m long) for tomato and were raised 23 cm high for lettuce. Drip irrigation tape (low flow, 1.6 cm diameter, 0.2 mm thick, and 20 cm emitter spacing) was placed on the center of the bed. For lettuce, black polyethylene mulch (0.03 mm; Berry Plastics Corporation, Evansville, IN) was laid over the pre-formed beds using a Model 2600 Raised Bed Mulch Layer (RainFlo Irrigation, East Pearl, PA).

For tomato, six different mulch types were evaluated: BioAgri Ag-Film (0.02 mm; BioBag, Palm Harbor, FL); BioTelo Agri (0.02 mm; Dubois Agrinovation, Waterford, ON, CAN); an experimental-nonwoven spunbond PLA (white 0.64 mm SB-PLA-10 in 2010, black 0.58 mm SB-PLA-11, and black 0.39 mm 75% SB-PLA+ 25% PHA in 2012; NatureWorks LLC, Blair, NE); black polyethylene, (0.03 mm; Berry Plastics Corporation, Evansville, IN); WeedGuardPlus (0.23 mm; Sunshine Paper Co. LLC, Aurora, CO); and, bare ground control. All mulch treatments were arranged in a split block design with four replications of high tunnel and
open field plots. Subplot size was 0.9 m wide x 4 m long. Seven six-week-old tomato seedlings of cultivar Celebrity were transplanted into each subplot using 60 cm in-row spacing and 1.8 m between-row spacing, and planting holes were hand punched into the mulched rows.

Six lettuce cultivars were evaluated in the same high tunnel and open field plots. The cultivars in 2010 and 2011 included Boston types Adriana and Ermosa; leaf types Green Star and New Red Fire; and Romaine types Coastal Star and Jericho. In 2012, Boston types Concept and Nevada; leaf types New Red Fire and Red Sails; and Romaine types Coastal Star and Valmaine were evaluated. Six-week-old seedlings were transplanted by hand into two staggered rows with in-row spacing of 30 cm and between-row spacing of 30 cm; spacing between beds was 1.8 m spacing center-to-center. Subplot size was 0.9 m wide x 4.3 m long.

In this study, high tunnels were constructed parallel to prevailing wind direction because wind gusts could exceed the ratings for the model. Ventilation of sidewalls only occurred when strong winds threatened high tunnel structures or when air temperatures inside the high tunnels threatened the health of the crops. High tunnels were erected in the same field location each year, and the plots assigned for each mulch treatment remained constant throughout the study. However, lettuce was rotated with strawberry within each main plot on an annual basis.


Fig. 1. Lettuce in raised beds, mulched with black polyethylene, transplanted by hand with 31 cm spacing between plants in two staggered rows and A, seedlings inside a high tunnel plot and B, mature heads in open field.
CHAPTER TWO
COMPARISON OF DISEASE OUTBREAKS IN ORGANICALLY-MANAGED
TOMATOES GROWN WITH DEGRADABLE MULCHES,
IN HIGH TUNNELS VS. OPEN FIELD SETTINGS
IN WESTERN WASHINGTON

Introduction

Tomatoes (Solanum lycopersicum, formerly Lycopersicon esculentum Mill) are high value crops that usually require warm growing conditions. However, some early maturing and cold-tolerant cultivars can be grown in western Washington, where the climate is mild and the growing season is short compared to other parts of the U.S. Most of the tomatoes grown in Washington are for the fresh market. Production in western Washington occurs in Clallam, Clark, Cowlitz, Grays Harbor, Island, Jefferson, King, Kitsap, Mason, Pierce, San Juan, Skagit, Thurston, Wahkiakum, and Whatcom Counties (4). According to the USDA National Agriculture Statistics Service (NASS), Washington produced 242 tons of organic tomatoes for fresh-markets from only 49 acres in 2008 and sales were valued at $301,577. These figures represent only a small portion of overall tomato production in the U.S., which in 2011, was 105,400 acres with sales valued at $1.3 billion, according to a NASS survey (50). Since locally-grown organic vegetables are highly sought after, a niche market for organic tomato growers exists in Washington. Given that the optimum temperatures for growing tomato are between 25 to 30°C (30), and the climate in western Washington is generally cool ranging 15 to 17°C from June to September (38), mulches and/or high tunnels need to be used in order to grow commercial tomatoes in the field successfully (22).
Plasticulture, the use of plastic in agriculture, includes protective structures like high tunnels, mulches, and tubing for drip irrigation. In Washington, plasticulture increases the cost of tomato production by approximately $159 per m², but net returns can improve by approximately $108 per m² (22). Studies show that protective structures can improve tomato fruit yield compared to open field production in some climates (41). Mulches also can be used to warm the soil, reduce weed germination and conserve water, and can be applied with relative ease when in the form of a film (34). Black polyethylene is the most common mulch because it is light weight, relatively less expensive than other films, and stretches easily over beds with machinery (34). Currently, mulches that are biodegradable are available commercially (see Chapter One).

Tomato plant health and yield are affected by mulching. Mulch color, specifically, can induce changes in leaf area index (21) especially of younger leaves (15), the total number of fruit (15; 36), and the severity of some plant diseases (35). Lops et al. (35) found that black mulch film reduced corky root on tomato caused by *Pyrenochaeta lycopersici* R. Schneider & Gerlach compared to white and green mulch film but not compared to bare ground; silver mulches reduced infections by tomato spotted wilt virus.

Tomato is susceptible to wide range of fungal and bacterial diseases which occur during various stages of phenology. Descriptions of symptoms of select tomato diseases in western Washington can be found in Chapter One. Foliar pathogens found on tomato under field conditions in western Washington (39) include *Alternaria tomatophilai* Sorauer (formerly *A. solani*), which causes early blight, and *Phytophthora infestans* (Mont.) de Bary the cause of late blight. Incidence of both of these diseases is reduced under high tunnel production (25; 33; 48; 53) because the pathogens are either rain-dispersed or require prolonged periods of leaf wetness to infect plant tissues and produce secondary sporangia or spores. Early blight develops more
readily on plants stressed by nitrogen deficiency (51); some growers, including those in western Washington, reduce nitrogen levels to promote reproductive growth (13) which in turn can make plants more susceptible to this disease. Almost all tomato cultivars grown in western Washington are susceptible to late blight. Further, populations of *P. infestans* in the region are complex and have resistance to the systemic fungicide, mefenoxam (16), which makes control by protectant fungicides challenging and expensive. In a previous study, different genotypes of *P. infestans* were identified in western Washington, i.e., US-7, US-8, US-11, and US-14 (16). Both A1 and A2 mating types were confirmed by the authors, although sexual recombination was not detected (16).

Soilborne pathogens that cause vascular wilts on field tomato in Washington (39) include *Fusarium oxysporum* f. sp. *lycopersici* (Sacc) Snyder & Hans., *Verticillium albo-atrum* Reinke et Berth, and *V. dahliae* Kleb. Damping-off of seedlings can be caused by *Pythium* Pringsh spp. and *Rhizoctonia solani* Kühn (39) while root damage can be caused by *Meloidogyne hapla* Chitwood (39). Stem lesions caused by *Sclerotinia sclerotiorum* (Lib.) de Bary may also cause plant collapse if the stem becomes wounded (39). *Pectobacterium carotovorum* (Jones) Hauben (syn. *Erwinia carotovorum* subsp. *carotovora* (Jones) Bergey) causes crop losses on potato in Washington (39) but can also infect tomato (47). In comparison, tomato grown under greenhouse production is more likely to be affected by a different spectrum of pathogens which thrive under high relative humidity and warm temperatures typical of protective structures. One common foliar pathogen in high tunnels and greenhouses in Washington (39) is *Fulvia fulva* Cooke (syn. *Cladosporium fulvum*), the cause of leaf mold. Tomato fruits and stems may also be affected by *Botrytis cinerea* Pers. ex Fr. (teleomorph *Botryotinia fuckeliana* Whetzel (39) which causes gray mold. Powdery mildew caused by *Leveillula taurica* (Lév.) Arnaud (anamorph: *Oidiopsis sicula*)
is present in tomato production in eastern Washington (39), but has not been detected in greenhouses or high tunnels in western Washington even though high tunnel production can favor powdery mildews such as has been observed for strawberry (56).

In addition, tomato pith necrosis caused by *Pseudomonas corrugata* Scarlett or *Pseudomonas* complex (11) is an emerging disease on tomato in many commercial growing areas of the world. Generally, field infections are favored by cool night temperatures and high relative humidity (44), and whenever excess nitrogen is applied to plants grown under mulches or in high tunnels (10; 37; 46). The tender tissues of transplants in the presence of abundant nitrogen and wounded tomato roots and stems favor infections (11). *P. corrugata* is a ubiquitous bacterium (11) considered mildly pathogenic because plants can recover after infection, but in severely infected plants, yields are reduced and some plants may die. Controlling soil populations of *P. corrugata* may prove to be difficult as this bacterium is primarily saprophytic, with broad metabolic activities, and may survive in bulk soil and the rhizosphere (2).

Research shows that other bacterial diseases like soft rot caused by *Pectobacterium carotovora* (Jones) Waldee (formerly *Erwinia carotovora*) can increase with the use of plasticulture. Onion bulb decay increased by 43% when crops were grown under black plastic compared to bare ground (24). The authors attributed this increase in bulb decay to higher soil temperatures, known to favor bacterial growth, because silver mulch, which reflects solar radiation, reduced bulb rot by 34% compared to the black plastic treatment.

Although the etiology of physiological leaf roll (PLR) on tomato has not been clearly defined, several contributing factors have been demonstrated. These include excess nitrogen and phosphorus deficiencies, prolonged periods of dry weather which can disrupt available water to roots, and starch accumulation in lower leaves due to heavy pruning of new growth (5; 54).
Further, PLR may be cultivar dependent and not solely reliant by determinate versus indeterminate growth, variable fruit production, or high tunnel and open field production systems (26).

Mulches and protective structures modify air and soil environments, and can affect plant diseases. However, mulches, biodegradable and otherwise, can also affect plant health. The effects of using biodegradable mulches, especially on root diseases and the survival of soilborne pathogens, need to be explored for tomato production. The purpose of this study was to evaluate naturally-occurring outbreaks of tomato diseases on organically-managed Celebrity plants grown with selected agricultural mulches in high tunnel and open field production systems in western Washington.

Materials and methods

Tomato cultivar. Celebrity is an all-purpose hybrid tomato cultivar that has mid-season maturity, cold tolerance, and a determinate growth pattern (14). It was originally distributed by PetoSeed/Seminis (Jim Myers, personal communication). Celebrity is a popular cultivar with desirable flavor, high yield and quality (49). The cultivar won the All-American Standard award in 1984 (14). In addition, Celebrity has some tolerance to Fusarium wilt (Race 1 and Race 2), Verticillium wilt (Race 1), and root knot nematode (9; 23; 57). Celebrity is resistant to a few of the common pathogens that affect tomato in western Washington as described above, and was selected for this study because it can be grown in various climates including the mild, marine climate of western Washington.

Field preparation. Four high tunnels (‘Solo’ model, Haygrove LTD, UK) each measuring 37 m long x 8 m wide x 3 m high were erected at Washington State University Mount Vernon
NWREC in mid-April and removed in late October for three consecutive tomato cropping cycles, from 2010 through 2012. Prior to planting tomato each year, soil was amended with organic fertilizer Par4 9-3-7 (North Pacific Ag Products, Portland, OR) at 89 kg N·ha\(^{-1}\) to row centers on 27 to 29 Apr in 2010 and 3 May in 2011. In 2012, organic fertilizer Proganic 8-2-4 (Wilbur Ellis, Wenatchee, WA) was broadcast applied at 89 kg N·ha\(^{-1}\) on 23 Apr. Flat beds (0.9 m wide x 37 m long) were shaped with an Allis-Chalmers Model G tractor (Allis-Chalmers Manufacturing Co., Milwaukee, WI) and low flow drip tape (1.6 cm dia, 0.2 mm thick, 20 cm emitter spacing) was laid on the top of each bed. Mulches were laid over the beds on 25 May in 2010; 12 and 24 May in 2011 in high tunnel and open fields, respectively; and, 11 May in 2012. The edges of the mulch were secured by placing them in a furrow along the bed widths, and furrows were backfilled with soil using a shovel.

Each of the three experiments consisted of four replications of high tunnel and open field plots with six mulch treatments arranged in a split plot design; mulch treatments were assigned as subplots, 0.9 m wide x 4 m long. The mulch treatments included: two commercially-available biodegradable mulches (BDMs) BioAgri (BA, 0.02 mm, BioBag, Palm Harbor, FL) and BioTelo (BT, 0.02 mm, Dubois Agrinovation, Waterford, ON, CAN) that are manufactured with Mater-Bi\(^{\circledast}\); one experimental-nonwoven spunbond (SB) poly(lactic) acid (PLA) mulch (white, 0.64 mm SB-PLA-10 in 2010, black, 0.58 mm SB-PLA-11 in 2011, and black, 0.39 mm SB-PLA(75%) + polyhydroxyalkanoates (PHA; 25%) in 2012; NatureWorks LLC, Blair, NE); one cellulose mulch control, WeedGuardPlus (WG, 0.23 mm, Sunshine Paper Co. LLC, Aurora, CO); one standard plastic mulch control, black polyethylene, 1.0 mil (0.03 mm) (BP, Berry Plastics Corporation, Evansville, IN); and, one no-mulch control (BG, bare ground).
Seeds of tomato cultivar Celebrity were sown in 72-cell plug trays on 19 and 30 Apr in 2010, and 4 and 19 Apr in both 2011 and 2012. The seedlings were used as the high tunnel and open field transplants after hardening off for one week outdoors prior to transplanting in the field. Seven six-week-old seedlings per mulch treatment were transplanted by hand into the high tunnels and open fields on 27 May and 3 June in 2010, 12 May and 31 May in 2011, and 21 May and 30 May in 2012, respectively. Plants were spaced 61 cm apart in the row, and between row spacing was 1.8 m. Dead seedlings resulting from mechanical injury were replaced during the first three to four weeks only, and were not replaced thereafter as transplants would not have had sufficient time to recover and grow comparably to surrounding plants. Irrigation was applied at 1.2 cm per week in 2010 and 2011, and 1.4 cm per week in 2012. Fertigation with Converted Organics 521 Fertilizer (Converted Organics of California LLC, Gonzales, CA) was initiated at transplanting and applied through the irrigation system at 11 kg N·ha⁻¹·day⁻¹ approximately every 10 days in 2010, 7.4 kg N·ha⁻¹·day⁻¹ approximately every seven days in 2011, and 2.5 kg N·ha⁻¹·day⁻¹ every seven days in 2012. The application times were changed to a seven-day cycle upon flower set in 2010 and 2011, and to a three-to-four day cycle in 2012. Fertigation occurred 12 times in 2010, 22 and 21 times in 2011 for high tunnel and open field plots, respectively, and 20 and 18 times in 2012 for high tunnel and open field plots, respectively. Approximately two to three weeks after transplanting, when plant heights reached 30 to 38 cm, plants were staked and trellised via a Florida weave system (55). Plants were pruned periodically so that suckers starting from the second lateral branch below the first flower set were removed, leaving one central leader.

Temperature monitoring. Soil temperature at five cm depth and air temperature were recorded at 15-minute intervals with a Hobo U-30 Weather Station (Onset Computer, Bourne,
MA) installed in the center of each mulch treatment in one high tunnel and one open field plot each year. The soil temperature sensors were installed 30 cm from mulch edge and 20 cm from the drip tape. Data were collected from 21 May to 8 Oct in 2010, 19 May to 28 Oct in 2011, and 21 May to 11 Oct in 2012. Soil moisture data collection was discontinued after 2010 due to inconsistent results, and therefore, were not used in this analysis.

**Disease, insect, and weed control.** The measures taken for disease, insect, and weed control are listed in Table 1. Most control measures were approved for organic tomato production. However, due to the severity of the late blight epidemic in 2012, some fungicides not registered for organic production were used. All applications were made according to the manufacturer’s directions.

**Disease assessments of plants and fruit.** Each week during the growing season, plots were visually rated for disease incidence and severity. Incidence was recorded as total number of plants affected and severity was recorded as a percentage, 0.0001 to 100%, of plant tissues affected per plot. If only one to fifteen leaves in a plot were symptomatic, severity ranged from 0.0001 to 0.5%. When one whole plant was affected by a disease, 15% severity was assigned. If all plants in a plot were affected but only the base or crown tissues were symptomatic then a 1 to 20% severity rating was designated. If all plants in the plot were affected, disease severity ratings reached 30 to 100%. Ratings were done at the same time each week to increase the likelihood of similar lighting for viewing. Symptomatic and non-symptomatic plants were also photographed. Weekly field ratings ceased on 27 Sept in 2010, 4 Oct in 2011, and 21 Sept in 2012, prior to final tomato harvests. In order to secure representative fruit yield for fair yield comparisons in 2011 and 2012, all tomato plant tissues with symptoms of late blight were recorded weekly and bi-
weekly but then removed with pruners to delay the progress of the epidemic. Treatment comparisons were thus by number of lesions, totaled over the entire season.

Comparisons of plant health were also made and the categories included healthy, weak and dead. Healthy plants had new growth, lush green leaves, and produced flowers and/or fruit at expected times. Weak plants were either stunted, chlorotic, wilted, or had delayed fruit set as compared to other plants. Dead plants were categorized by primary symptoms of rot or wilt, and select plants were destructively sampled either during or at the end of the growing season when all plants parts including roots were assessed for disease.

Tomato fruit were harvested by hand throughout the season. Fruit was harvested when it was at least 75% red. Plots were harvested once or twice a week as needed. Fruit was harvested from high tunnel plots 2 Sep to 19 Oct (98 to 145 DAT) in 2010; 6 Sep to 21 Oct (116 to 161 DAT) in 2011; and, 30 Aug to 8 Oct (100 to 139 DAT) in 2012. Fruit from open field plots were harvested 2 Sep to 6 Oct (91 to 125 DAT) in 2010; 22 Sep to 19 Oct (114 to 141 DAT) in 2011; and, 24 Sep to 4 Oct (116 to 126 DAT) in 2012. Harvested fruit were visually rated as diseased or healthy. Diseased fruit were distinguished by rot, discoloration, or sporulation but did not include secondary infections due to blossom end rot or cracking. Disease incidence was recorded as noted above but severity was rated on a per fruit basis. Rot, discoloration, sclerotial formation and skin separation, all discreet symptoms, were rated separately using a 0 to 4 scale. Fruit were rated zero when no symptoms were present; one when 1 to 25% of fruit was affected; two for 26 to 50% severity; three for 51 to 75% severity; and, four when 76 to 100% of the fruit was affected.

Isolate characterization and identification. Diseased plant tissues, obtained during weekly field ratings or at harvest, were used to obtain isolates of the potential pathogens via sterile
technique. (Pathogenicity is currently being confirmed via Koch’s postulates in the greenhouse and identification is anticipated to be confirmed with PCR, followed by sequence analysis upon completion of those trials, estimated around December 2012.)

Tissues were plated directly or after surface disinfesting in 70% EtOH for 30 sec, followed by washing in a 10% NaOCl solution (30 s for leaves, 60 s for stems and roots), and rinsing with sterile water, depending on the suspected disease. The tissues were either (i) placed in a moist chamber with sterile water or 1% NaOCl, (ii) ground with a mortar and pestle then streaked onto nutrient broth yeast (NBY) medium (3), water agar, or King’s B agar (3) media, or (iii) plated onto half-strength potato dextrose agar (1/2 PDA) or rye agar media as described by Erwin and Ribeiro (20) except glucose was excluded. For example, when suspected late blight-affected tissues were removed, surface disinfection was not used as sporangia of *P. infestans* are more successfully isolated from the surface of sporulating lesions kept in a moist chamber. All isolates of *P. infestans*, Pi.Sl.2010, Pi.Sl.2011, and Pi.Sl.2012 were maintained on rye agar, stored at 16°C, and transferred to a new agar plate every four weeks. In 2011 a few plants exhibited symptoms of early blight and leaf tissues were removed from the field and then directly transferred to a moist chamber and stored at ambient light and temperature for two weeks. Additionally, sclerotia formed on culture media containing symptomatic tissues, were transferred to an envelope, air dried over-night in a laminar flow hood, and kept at 4°C in a desiccator. The identification of fungal isolates was by visual characterization of colonies and measurement of spore size and mycelial structures by using a light microscope at 400 X magnification.

When tomato pith necrosis was suspected, tissues were surface disinfected prior to cutting stems and removing pith tissue. In 2011 all tomato plants exhibiting symptoms of this
disease were destructively sampled from affected high tunnel and open field plots. For comparison, one healthy plant was also randomly selected from each affected plot. Roots from all destructively sampled plants were cut 1 cm above soil line, washed, weighed (fresh and dry weight), and lesions counted. Symptomatic areas from select roots with lesions were surface disinfected as above, and incubated on water and PDA media. Stems from symptomatic plants were also surface disinfected prior to removing pith tissues with sterile forceps, and then homogenized before the liquid was streaked onto water agar medium. Symptomatic plants were incubated in moist chambers, the plant extracts streaked onto agar plates kept at room temperature, and the cultures were observed for sporulation or single colony production. Discreet colonies on water agar plates were transferred to King’s B and NBY media. Each bacterial isolate was stored in 30% w/v glycerol vials kept at -80°C. *P. carotovorum* was suspected to have caused plant collapse in one plant from a BioAgri subplot in the open field in 2012. Six isolates were obtained from macerated tissues and tested for pectinolytic capabilities on CVP, Hilbrand’s A and C agar media (3), and designated as Pbc.2012-1 through Pbc.2012-6. Selected isolates that tested positive for pectinolytic activity were assessed for oxidase potential (32).

Isolates of *P. infestans* and *B. cinerea* were not tested for pathogenicity because these pathogens have already been well documented on tomato. However, isolates of bacteria were tested for pathogenicity. Selected cultures were grown overnight on NBY agar medium before a single colony was transferred to a 15 ml culture tube with NBY broth for incubation at 28°C overnight. Cells were washed and pelleted, then diluted to $1.0 \times 10^8$ cfu·ml$^{-1}$. In a first experiment, Celebrity tomato seedlings (five-to-six leaf stage) were grown in a one gallon pot and water stressed two days prior to inoculation. Plants were inoculated with isolate BDMPc.Sl.2011 by puncturing the stem twice at the second or third leaf axil with a sterile
needle, then slowly dripping 10 µl of $1.0 \times 10^8$ cfu·ml$^{-1}$ solution onto the fresh wound. Plants were wrapped in clear plastic to create high relative humidity conditions following the absorption of inoculum, and incubated at 21°C on a greenhouse bench for 48 hours. In a second and third experiment, isolates BDMPc.Sl.2011, CvPc.Sl.2012-1, CvPc.Sl.2012-2, CvPc.Sl.2012-3, Pbc.2012-3, and Pbc.2012-5 were tested on Celebrity seedlings. Plants were inoculated at the three-to-four leaf stage, but otherwise according to the above inoculation procedure. Each plant was transferred to a 10 cm$^2$ pot, wrapped in a clear plastic bag for four days, grown on a greenhouse bench at 21°C with a 14-hr photoperiod, and sampled upon the development of symptoms. The identities of bacterial isolates BDMPc.Sl.2011, Pbc.2012-3, and Pbc.2012-5 were assigned via bioassays on selective agar including King’s B, CVP, and Hildebrand’s A and C media (3) and growth at 37°C. (PCR and sequence analysis of isolates obtained from symptomatic tissues is anticipated to be completed when the inoculated plants, now growing in the greenhouse, are mature and expressing symptoms.)

Data analysis. Data regarding disease incidence during weekly field ratings or at harvest were subjected to analysis of variance using the PROC MIXED procedure of SAS v. 9.2 (SAS Institute Inc., Cary, NC) according to the Satterthwaite method (43) for assessing degrees of freedom. Least squared means was used to obtain mean separations. Rank transformations were assigned based on the range method described by Kirk (31) for the cases where the data did not meet assumptions of normality and homogeneity of variance.

Results

Environmental monitoring. Average soil temperatures over the growing season were greater in 2010 compared to 2011, and greater in 2012 compared to 2011. Soil temperature was
greater in the high tunnel plots than in the open field plots, and greater under the dark colored versus under light colored mulches (Table 2). Soil temperatures were also higher under black polyethylene mulch than under BioAgri and BioTelo. In 2010, soil temperatures under BioAgri and BioTelo were 0.9 and 0.5°C, respectively, cooler than black polyethylene in high tunnels, and 1.2 and 0.9°C, respectively, cooler than under black polyethylene in open field plots. In 2011, soil temperatures were again cooler under BioAgri and BioTelo than black polyethylene by 0.8 and 1.0°C, respectively, in high tunnels, and by 0.2 and 0.6°C, respectively, in the open field. In 2012, soil temperatures were reduced under BioAgri and BioTelo compared to black polyethylene by 1.4 and 1.3°C, respectively, in high tunnels and by 0.2 and 0.6°C, respectively, in open fields. Interestingly, soil temperatures recorded for bare ground were generally greater, ranging from 0.4 to 1.0°C, than for soil under WeedGuardPlus in the high tunnels in 2010, 2011 and 2012 (Fig. 2). In the open field, soil temperature under WeedGuardPlus was cooler than the bare ground in 2010 by 0.3°C and in 2012 by 1.4°C, but was 0.1°C warmer than bare ground in 2011.

Average air temperature was also higher in 2010 compared to 2011, but similar between 2011 and 2012 (Table 2). Higher air temperatures were recorded in the high tunnel than in the open field. However, each year, the early and late season air temperatures were slightly cooler in the high tunnels than in the open fields: 0.04 to 0.2°C cooler in 2010; 0.1 to 4.4°C cooler in 2011, and 0.3 to 2.3°C in 2012. This seasonal trend resulted in lower air temperature recordings inside high tunnels than in the open field 2.0% of the growing season in 2010, and 29.0% and 28.6% of the season in 2011 and 2012, respectively (Fig. 3). For the remainder of the growing season, air temperatures inside the high tunnels always exceeded open field air temperatures, and by a maximum of 2.9, 6.5, and 8.7°C in 2010, 2011, and 2012, respectively.
Disease outbreaks. Late blight and PLR were observed all three years in both production systems and among all mulch treatments, including the bare ground controls. The onset of late blight occurred in the high tunnels and the open fields on the same dates: 19 Aug (84 and 77 DAT) in 2010, 29 Aug (109 and 90 DAT) in 2011, and 26 Jul (65 and 55 DAT) in 2012, respectively. It is important to note that transplanting dates were delayed in open field relative to high tunnel plots by seven days in 2010, 19 days in 2011, and 10 days in 2012 due to weather conditions. In the week prior to disease onset, air temperature averaged 19.2, 16.7, and 15.8°C; relative humidity was 72.2, 83.3, and 84.4%; and, leaf wetness accumulation was 33, 65, and 51 hours for 2010, 2011, and 2012, respectively. Precipitation during the week prior to disease onset only occurred in 2012, when 1.5 cm was recorded. Late blight epidemics progressed until 27 Sept in 2010, 21 Sept in 2011, and 13 Sept in 2012.

The potential for a very severe epidemic of late blight occurred in 2012, but an aggressive sanitation and preventive fungicide spray program successfully delayed disease progress. Relative to 2010 and 2011, the onset of late blight in 2012 occurred 19 and 44 DAT earlier in high tunnels and 21 and 35 DAT earlier in open fields, respectively. Within one week after disease onset, severity ranged from 0.1 to 15% in open field plots, but in high tunnel plots severity only ranged from 0 to 0.001%. The sporangia were typically limoniform and size averaged 43.5 x 71 µm. (Isolates, Pi.Sl.2010, Pi.Sl.2011, and Pi.Sl.2012, will be identified via molecular analysis using restriction fragment length polymorphisms to characterize them into specific U.S. classifications, and results are expected by February 2013.)

The incidence of late blight was significantly ($P = 0.0006$, $P = 0.0019$, and $P = 0.0034$, respectively) higher in open fields compared to high tunnels all three years, but not significantly ($P = 0.9713$, $P = 0.4759$, and $P = 0.1078$) different among mulch treatments in 2010, 2011, or
2012, respectively (Table 3). Late blight proved to be the most severe in open field plots in 2010 when sanitation measures and preventive fungicides were not employed, allowing recurrent sporulation and disease spread. In 2010, nine out of 24 open field plots (37.5%) were rated ≥ 50% severity. Of these plots one plot reached 80% severity (Fig. 4A), two reached 75%, two reached 60%, and four reached 50% during the peak of the outbreak. During this same period of time, late blight in high tunnel plots only ranged from 0.001 to 1% severity (Fig. 4B). Late blight incidence increased from 24 to 48 lesions total in open fields during the first two weeks of September in 2011 when monthly air temperatures averaged 16.1°C, relative humidity averaged 75.5% but no rain fall was recorded. During this time period only nine lesions total were recorded in the high tunnels, however. Late blight was less severe in 2011 and 2012 compared to 2010, but diseased tissue was continually removed during the latter two years.

PLR was significantly ($P < 0.0001$ and $P < 0.0001$) greater for BioAgri, BioTelo, and black polyethylene mulch treatments than for spunbond-PLA-10, SB-PLA-11, WeedGuardPlus, or bare ground treatments in open field plots in 2010, and in both production systems in 2011, respectively (Table 4). In 2012, PLR was significantly ($P < 0.0001$) greater for BioTelo and black polyethylene compared to other mulch treatments. PLR was first observed 43, 48 and 45 DAT in the high tunnels, and 36, 29, and 35 DAT in the open field plots, in 2010, 2011 and 2012, respectively. PLR occurred first on older leaves and was rarely observed in new growth. There was a significant interaction between mulch and production system in 2010, but not in 2011 or 2012. In 2011 severity ratings (Fig. 5) for PLR were significantly ($P < 0.0001$) greater in the high tunnel production system versus the open field. In 2012, no significant difference in PLR severity was detected by production system. In 2010 and 2011 PLR severity peaked around the warmest times of the growing season, late-Aug to early-Sept, but during this time in 2012,
PLR severity was reduced inside the high tunnels than in the open fields. Air temperatures recorded during these weeks in 2012 ranged from 13.1 to 14.8°C, and newly expanded leaves produced by plants inside the high tunnels were not affected by PLR. However, plants in the open fields produced little to no new growth regardless of mulch treatment, and therefore PLR severity did not decrease over time in 2012. Weeks prior to this cool weather in 2012, air temperatures ranged from 14.7 to 21.1°C.

Tomato pith necrosis was first observed in 2011 on nine out of 336 plants (2.7%); eight of the affected plants (89%) were from open field plots but mulch type did not appear to affect incidence of this typically soilborne bacterial disease. Disease onset occurred mid-season, about the time of fruit set. Symptoms included black streaking along stem and petioles, bright yellow lesions that extended from leaf margin to midveins, and the formation of adventitious roots. The symptoms spread gradually and acropetally (Fig. 6). Two plants in the open field collapsed due to the disease but severity remained constant for other affected plants even when air temperatures dropped from 15.9°C in early- to mid-September to 9.7°C in early October. A bacterium that formed cream-colored colonies with irregular margins on NBY agar media was isolated from pith tissues of a Celebrity plant grown in a high tunnel under SB-PLA-11 mulch. This isolate, BDMPc.Sl.2011, was gram negative, non-fluorescent on King’s B medium, and grew at 37°C—three characteristics typical of \textit{P. corrugata}. In 2012, three suspected isolates of \textit{P. corrugata}, CvPc.Sl.2012-1, CvPc.Sl.2012-2, and CvPc.Sl.2012-3, were obtained from a single Celebrity plant in an adjacent separate trial evaluating tomato cultivar performance.

In 2012, one plant wilted within six days of observation and the stem was completely black (Fig. 7A), similar to the symptoms of late blight, but no sporangia were noted. The plant was cut at the base and green bacterial ooze dripped from the stem. It was noted that infection
likely occurred at a pruning site. A pungent rotting smell was also noted. Pith tissue was
removed from the periphery of black rotted areas (Fig. 7B and C), and streaked colonies on NBY
were white, orange, or cream colored with smooth margins. Two of the six isolates showed
pectolytic activity. Isolate Pbc.2012-3 caused pitting on Hildebrand’s A agar and Isolate
Pbc.2012-5 liquefied and turned Hildebrand’s C agar from blue to green (Fig. 7D and E).

According to seed distributors, Territorial Seed Company (Cottage Grove, OR) in 2010
and 2012 and Harris Seeds (Rochester, NY) in 2011 (6), tomato cultivar Celebrity F1 is resistant
to Verticillium wilt (race 1) as well as to several other diseases as already mentioned in the
Introduction. Verticillium wilt was noted on only one plant in the SB-PLA-11 mulch treatment
within a high tunnel plot in 2011. V. tricorpus was isolated from root and fruit tissues of the plant
but these tissues were also infected by P. corrugata. V. tricorpus was never isolated from open
field tomato plots, including other plants with symptoms of tomato pith necrosis. In a
preliminary study in the greenhouse, tomato cultivar Celebrity was inoculated with V. tricorpus
and BDMPC.Sl.2011 separately, and another set of plants was co-inoculated with both isolates by
dipping roots in both conidial and bacterial suspensions. Disease symptoms did not appear in any
of the plants and neither isolate was recovered from stem or petiole tissue. In a separate study,
soil samples were collected from tomato subplots in 2011. V. dahliae, a pathogen of tomato was
identified in soils from high tunnels and open fields (data not shown). Although soilborne
pathogens of tomato were identified, root and vascular diseases were not observed.

Early blight caused by A. tomatophilai was suspected in 2011. The foliage had gray
lesions with concentric rings; however, yellow margins were faint (Fig. 8). Several attempts were
made to isolate the pathogen from symptomatic tissues at two separate laboratories but both
attempts were unsuccessful. In 2010, F. fulvia was isolated from another tomato cultivar, in an
adjacent study within a high tunnel. Typical symptoms of leaf mold caused by *F. fulvia* were not observed on Celebrity in either production system in any year. Root rot diseases caused by soilborne pathogens were also not evident any year and no galls suggesting infections by root knot nematode were ever observed. In 2011, when plants were destructively sampled, only small-sunken gray or brown lesions had developed on roots. Two isolates of *Colletotrichum* spp. were obtained from two bare ground control treatments but the isolates were not identified or confirmed for pathogenicity.

**Tomato diseases at harvest and yield.** Only three diseases affected tomato fruit in 2011 and 2012: late blight, gray mold, and white mold (Fig. 9). Gray mold was observed in both production systems in 2010 and 2011, and late blight was noted on tomatoes from both systems in 2011, whereas white mold was only observed in both systems in 2012. Disease incidence did not exceed more than 11 tomato fruit in any year for these three diseases (Table 5), thus analysis of disease as a function of mulch was not possible. Although disease incidence was low, several fruit were deemed unmarketable due to blossom end rot and cracking (data not shown). One isolate of *B. cinerea*, Bc.Sl.2012, was obtained from an infected fruit and cultured on PDA medium.

Dark mulches increased total yield compared to light colored mulches in 2010 and 2011, but in 2011 tomatoes grown on bare ground yielded similar numbers and weights to fruits harvested from plants grown under dark mulches (Table 6). In 2010 and 2011, BioAgri produced significantly (*P* = 0.0083 and *P* = 0.0036) more fruit and total fruit weight was significantly (*P* = 0.0036 and *P* = 0.0231) greater than SB-PLA-10 in 2010 and SB-PLA-11 in 2011, respectively. In 2012, total number and weight of fruit harvested was significantly (*P* = 0.0003 and *P* <
0.0001, respectively) greater in the high tunnels compared to the open fields, but not significantly different by mulch.

**Greenhouse pathogenicity tests with** *P. corrugata*. Isolate BDMPc.Sl.2011 was not pathogenic on inoculated seedlings of Celebrity in the first greenhouse study, and inoculated seedlings including stems and leaves never developed symptoms. One bacterium was isolated from pith tissue of one inoculated plant. This bacterium produced colonies with smooth margins on NBY agar, was gram positive, and did not grow at 37°C. (The second and third greenhouse study is ongoing, and upon completion, each bacterial isolate is anticipated to be characterized via biochemical assays listed in materials and methods and identified to species via PCR and sequence analysis.)

**Discussion**

**Disease outbreaks.** Late blight is an annual threat to tomato production in western Washington (19) because of the mild environmental conditions with periods of rain, two conditions that are conducive for disease. In this study, late blight was not affected by mulch type but incidence was modified by production system, as was expected based on other studies at the field site (25; 27). Previous reports also show that protective structures reduce leaf wetness, a key component of disease (see Chapter One). In 2010 and 2011, fungicide sprays used to control late blight were only applied to open field plots, but even so, plants in the open fields still had significantly higher incidence of late blight than plants in the high tunnels both years. In 2012 one single protective spray was mistakenly also applied to young plants inside the high tunnels early in the season, but late blight incidence was still lower on the new growth of these plants when compared to new growth in the open field. Many high tunnel models have endwalls, and if
kept closed these structures trap additional heat, reduce air flow, and raise relative humidity, similar to greenhouses (7; 28). If not managed properly, high tunnels like greenhouses, can create favorable environments for *P. infestans* to infect plants when air temperatures are around 15 to 23°C (8). The ‘Solo’ high tunnel model used in this study was open-ended, allowing passive ventilation to dry plant tissues at all times, but issues such as condensation, morning fogs, and ceiling leaks may explain late blight incidence in high tunnels, even though incidence was low all three years. Indeed, this study documented many late blight lesions occurring in the high tunnels where plants were located near the openings and in places where cooler temperatures and condensation were more likely. Other studies have shown that microenvironments exist inside high tunnels including slightly cooler air temperatures (2 to 3°C) near the ends of open-ended high tunnels (52).

Mulch type did affect PLR, and dark mulches in particular increased the severity of PLR compared to light-colored mulches. Although in the case of PLR there may be an advantage to using light-colored mulch, weed control could become problematic. The white experimental SB-PLA-10 mulch used in this study was not effective at reducing weed seed germination. Higher soil temperatures under the dark mulches (BioAgri, BioTelo, and black plastic) likely induced some heat stress during warm periods, and may have resulted in more evapotranspiration compared to the other mulch treatments. The higher temperatures under dark mulches could have also induced more vegetative growth compared to lighter mulches. Extra vegetation would have required more pruning to allow sufficient air flow, and this practice could have resulted in starch accumulation in lower leaves, especially for Celebrity, a vigorous growing tomato. Water stress and excess pruning tend to exacerbate PLR (5; 54). It should be noted that PLR has not been associated with yield loss (5).
Root and vascular diseases, which may be inhibited or promoted by changes in soil temperature, were not noted during weekly field ratings or during destructive sampling in 2011 and 2012. Some root diseases were expected since dark colored mulches can raise soil temperature (17; 21) thereby favoring growth of some pathogens like *Fusarium* spp., which grow optimally when air and soil temperatures reach 28°C (29). Although Celebrity is purported to be resistant to *F. oxysporum* f. sp. *lycopersici* Race 1 and Race 2, other cultivars with the same resistance, e.g. Floradade, used in commercial fresh-market production, are susceptible to race 3 (42). Soil temperatures in western Washington in 2010 to 2012 never reached 28°C regardless of mulch treatment so it is not surprising that *F. oxysporum* f. sp. *lycopersici* race 3 was not observed. Only *Colletotrichum* spp. were isolated from root lesions when plants were destructively sampled. However, it is likely that the two *Colletotrichum* isolates recovered are not pathogenic because Dillard and Cobb (18) report that *C. coccodes* commonly colonizes tomato roots during senescence, and root lesion severity is highest postharvest. Also, harvested fruit were not affected by anthracnose in any year, as would be expected for a pathogenic isolate. Occasionally fruit were affected by late blight, or by white or gray mold, but the incidence was too low to deduce whether mulch affected number of diseased fruit. Also, signs of *F. fulva* were not observed on Celebrity grown with any agricultural mulch or production system any year. In 2011, various tomato farms in Whatcom and King Counties in western Washington with high tunnels without forced air ventilation were affected by *F. fulvia*. Plants within these high tunnels were closely spaced and some of the structures where longer or lower than the Solo style erected for WSU NWREC’s BDM field experiment. The most severe case of leaf mold observed during the farm tours was noted in a high tunnel where endwalls were not open.
Mulches can reflect sunlight. Decoteau (15) showed that white and black mulch reflect 46 and 6% of sunlight, respectively, in the form of blue light (400 to 500 nm) from a distance of up to 10.6 cm from the mulch surface. Thus, mulches could affect incidence of stem lesions caused by pathogens sensitive to blue light, e.g. *B. cinerea* (40). Granted, this energy transfer is reduced as plants grow and shade the mulch (17). Stem lesions caused by *B. cinerea* were never noted any year, or among any treatment including bare ground, however.

In 2011, 2.7% of plants from both high tunnel and open fields were affected by tomato pith necrosis, a disease which has been associated with cool evening temperatures, excess nitrogen applications, high tunnels, and plastic mulch (10; 37; 46). Although plants with tomato pith necrosis did not appear to be affected by mulch type, too few plants were affected to statistically analyze effects of mulch on the development of this disease. However, eight out of the nine affected plants were located in open field plots, suggesting that the cooler evening temperatures could have contributed to the higher incidence in the open field. In addition, organic fertilizers typically release nitrogen slowly over time, and in 2011 tomato plants could have been more susceptible to *P. corrugata* if a release of nitrogen from previous applications caused tissues to grow rapidly and become vulnerable. Growers who apply excess nitrogen to improve yields (1) may be at the highest risk for tomato pith necrosis outbreaks since tender tissues are more prone to infection. Only recommended rates should be applied, especially when utilizing biodegradable and polyethylene mulches or high tunnels (10; 37; 46). One report suggests that *P. corrugata* is seedborne (12). Therefore, the diseased plants could have arisen from infected seed. Further studies evaluating the survival of *P. corrugata* under various mulches may prove beneficial since this bacterium has been associated with plant disease (44),
including stem and rootlet infections on tomato (45), and the ability to utilize various carbon sources (11).

No important differences in tomato diseases as affected by the mulch treatments were observed in this study, except for the difference in susceptibility to physiological leaf roll. Celebrity grows well in western Washington and the cultivar was not affected by some soilborne pathogens and potentially to some foliar pathogens (*Alternaria* sp. and *F. fulva*) common in the region. This study also confirms that high tunnels can reduce the amount of fungicides required to control late blight and demonstrated that in our region high tunnels can extend the tomato growing season for up to two weeks.
Literature cited


   zone temperature and on the manifestation of tomato spotted wilt symptoms and yield of 
   tomato. Scientia Horticulturae 114:90-95.

   reduction in incidence with foliar applied fungicides. Plant Disease 81:1439-1442.

   agriculture-screening for horizontal resistance to late blight in tomato. Pages 1-12. 
   Organic Farming Research Foundation.

    Phytopathological Society, St. Paul, Minnesota.


    producing high-tunnel tomatoes in western Washington. Washington State University 
    Extension Fact Sheet: (in press; to be published online).

    increases yield, but mulch color has no effect. HortScience 35(7):1213-1214.

    diseases and new research in plasticulture. Pages 138-140. in: 2011 New England 


TABLE 1. Disease and pest control application rates and schedules used on tomatoes grown under high tunnel and open field production systems in experimental trials near Mount Vernon, WA.

<table>
<thead>
<tr>
<th>Year</th>
<th>Target problem</th>
<th>Product applied</th>
<th>Rate applied per hectare</th>
<th>Dates applied</th>
<th>High tunnel DAT&lt;sup&gt;p&lt;/sup&gt;</th>
<th>Open field DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>blossom end rot</td>
<td>Organic Bio Link Cal Plus&lt;sup&gt;q&lt;/sup&gt;</td>
<td>4.7 liters</td>
<td>8 and 22 Sep</td>
<td>104 and 108</td>
<td>97 and 111</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>blossom end rot</td>
<td>Organic Bio Link Cal Plus</td>
<td>2.2 liters</td>
<td>10 Jun to 16 Sept</td>
<td>27 to 126&lt;sup&gt;r&lt;/sup&gt;</td>
<td>10 to 108&lt;sup&gt;r&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>blossom end rot</td>
<td>Organic Bio Link Cal Plus</td>
<td>4.7 liters</td>
<td>10 Aug to 4 Oct</td>
<td>80 to 133&lt;sup&gt;s&lt;/sup&gt;</td>
<td>71 to 124&lt;sup&gt;s&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>flea beetle</td>
<td>Entrust Naturalyte Insect Control&lt;sup&gt;t&lt;/sup&gt;</td>
<td>140 g</td>
<td>18 Jun</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>2011</td>
<td>flea beetle</td>
<td>PyGanic Crop Protection EC 5.0 ii&lt;sup&gt;u&lt;/sup&gt;</td>
<td>140 g</td>
<td>11 Jul</td>
<td>41</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>aphids</td>
<td>PyGanic Crop Protection EC 5.0 ii</td>
<td>4.9 kg</td>
<td>11 Jun and 4 Aug</td>
<td>15 and 69</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>aphids</td>
<td>PyGanic Crop Protection EC 5.0 ii</td>
<td>4.9 kg</td>
<td>11 Jun</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>aphids</td>
<td>Entrust Naturalyte Insect Control</td>
<td>175.2 g</td>
<td>8 Aug</td>
<td>86</td>
<td>69</td>
</tr>
<tr>
<td>2012</td>
<td>aphids</td>
<td>PyGanic Crop Protection EC 5.0 ii</td>
<td>9.1 kg</td>
<td>23 Jul and 4 Sep</td>
<td>62 and 115</td>
<td>53 and 107</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>thrips</td>
<td>Entrust Naturalyte Insect Control</td>
<td>140 g</td>
<td>9 Jul</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td>2012</td>
<td>thrips</td>
<td>Entrust Naturalyte Insect Control</td>
<td>175.2 g</td>
<td>23 Jul</td>
<td>62</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>late blight</td>
<td>Agri Star Nucop 50 WP&lt;sup&gt;v&lt;/sup&gt;</td>
<td>3.5 kg</td>
<td>20 Aug and 3 Sep</td>
<td>79 and 92</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>late blight</td>
<td>Nordox 75 WG&lt;sup&gt;w&lt;/sup&gt;</td>
<td>2.2 kg</td>
<td>22 Jul</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>late blight</td>
<td>Agri Star Nucop 50 WP</td>
<td>3.5 kg</td>
<td>11 and 26 Aug, 1, 7, and 14 Sep</td>
<td>72, 87, 93, 99, and 106</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>late blight</td>
<td>Agri Star Nucop 50 WP</td>
<td>3.5 kg</td>
<td>27 Jun and 16 Jul&lt;sup&gt;x&lt;/sup&gt;</td>
<td>36</td>
<td>27 and 46</td>
</tr>
<tr>
<td>2012</td>
<td>late blight</td>
<td>Bravo Weather Stik&lt;sup&gt;y&lt;/sup&gt;/Curzate 60 DF&lt;sup&gt;z&lt;/sup&gt;</td>
<td>1.7 liters and 224 g</td>
<td>27 Jul and 3, 10 and 17 Aug</td>
<td>57, 64, 71 and 78</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>late blight</td>
<td>Bravo Weather Stik</td>
<td>1.7 liters</td>
<td>24 Aug</td>
<td>85</td>
<td></td>
</tr>
</tbody>
</table>

<sup>p</sup>Days after transplanting (DAT) six-week-old tomato ‘Celebrity’ seedlings.

<sup>q</sup>Organic BioLink<sup>®</sup> Cal Plus (Westbridge, Vista, CA) was applied through irrigation.

<sup>r</sup>Applications were made once per week from 10 Jun to 16 Sept in 2011.

<sup>s</sup>Calcium was applied weekly from 10 Aug to 2 Oct in 2012, but increased to twice a week during late Aug.

<sup>t</sup>Entrust<sup>®</sup> Naturalyte Insect Control (Dow AgroSciences LLC, Indianapolis, IN) is certified for organic production.

<sup>u</sup>PyGanic<sup>®</sup> Crop Protection EC 5.0 ii (McLaughlin Gormley King Co., Minneapolis, MN) is certified for organic production.

<sup>v</sup>Agri Star<sup>®</sup> Nucop 50 WP (Albaugh Inc., Akeney, IA) which is allowed in organic tomato production.

<sup>w</sup>Nordox<sup>®</sup> 75 WG (Nordox AS, Oslo, Norway) was sprayed for fungicide rotation is certified for organic production.

<sup>x</sup>Agri Star Nucop 50 WP was only applied to the high tunnels on 27 June in 2012.

<sup>y</sup>Bravo Weather Stik (Syngenta Crop Protection LLC, Greensboro, NC) is not certified for organic production.

<sup>z</sup>Curzate 60DF (DuPont, Wilmington, DE) is not certified for organic production.
TABLE 2. Average soil and air temperatures, mid-May to early-October, for five agricultural mulches and bare ground with tomato cultivar Celebrity in an experimental field trial comparing high tunnel and open field production near Mount Vernon, WA.

<table>
<thead>
<tr>
<th>Year</th>
<th>Mulch</th>
<th>Average soil temperature(^u), °C</th>
<th>Average air temperature(^v), °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High tunnels(^x)</td>
<td>Open fields</td>
</tr>
<tr>
<td>2010</td>
<td>BA</td>
<td>18.9</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>BG</td>
<td>18.7</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>BP</td>
<td>19.8</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>BT</td>
<td>19.3</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>SB-PLA-10(^z)</td>
<td>18.7</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>WG</td>
<td>18.3</td>
<td>16.7</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td><strong>19.0</strong></td>
<td><strong>17.7</strong></td>
</tr>
<tr>
<td>2011</td>
<td>BA</td>
<td>17.1</td>
<td>17.1</td>
</tr>
<tr>
<td></td>
<td>BG</td>
<td>17.2</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>BP</td>
<td>17.9</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>BT</td>
<td>16.9</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>SB-PLA-11(^z)</td>
<td>16.9</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>WG</td>
<td>16.2</td>
<td>16.1</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td><strong>17.0</strong></td>
<td><strong>16.7</strong></td>
</tr>
<tr>
<td>2012</td>
<td>BA</td>
<td>17.5</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>BG</td>
<td>17.9</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>BP</td>
<td>18.9</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>BT</td>
<td>17.6</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>SB-PLA-12(^z)</td>
<td>18.2</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>WG</td>
<td>17.2</td>
<td>15.9</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td><strong>17.9</strong></td>
<td><strong>17.0</strong></td>
</tr>
</tbody>
</table>

\(^u\) Average soil temperatures obtained from 15-minute interval readings by sensors located 5 cm deep in the center of a 0.9 m wide x 4 m long mulch treatment plot, and attached to a Hobo U-30 Weather System (Onset Computer, Bourne, MA).

\(^v\) Average air temperatures calculated from daily averaged temperatures as recorded in 15-minute intervals by sensors attached to a Hobo U-30 Weather System (Onset Computer, Bourne, MA) placed in the center of a high tunnel and open field row (0.9 m wide x 37 m long).

\(^w\) Mulches include commercially available biodegradable mulches BioAgri, 0.02 mm (BioBag, Palm Harbor, FL) and BioTelo, 0.02 mm (Dubois Agrinovation, Waterford, ON, CAN); one cellulose mulch, WeedGuardPlus, 0.23 mm (Sunshine Paper Co. LLC, Aurora, CO); one standard plastic mulch control, black polyethylene, 0.03 mm (BP; Berry Plastics Corporation, Evansville, IN); and, one bare ground treatment.

\(^x\) High tunnels (‘Solo’ model, Haygrove LTD, UK) were covered with a single layer of 0.15 mm clear polyethylene (37 m long x 8 m wide x 3 m high).

\(^y\) --- indicates that air temperatures were not recorded for individual mulch treatments.

\(^z\) Experimental spunbond poly(lactic) acid (SB-PLA) mulch was modified annually: 2010 colored white, 0.64 mm, 2011 colored black, 0.58 mm, and 2012 colored black, 0.39 mm and a 75:25 mix of PLA:PHA; PLA = poly(lactic)acid and PHA = polyhydroxyalkanoates (NatureWorks LLC, Blair, NE).
TABLE 3. Area under disease progress curve (AUDPC) values in 2010, and mean number of late blight lesions in 2011 and 2012 for tomato cultivar Celebrity grown with five agricultural mulches and bare ground in experimental field trials comparing high tunnel and open field production near Mount Vernon, WA.

<table>
<thead>
<tr>
<th>Location</th>
<th>2010 AUDPC</th>
<th>2011 Mean no. lesions</th>
<th>2012 Mean no. lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>High tunnel</td>
<td>0.01 b</td>
<td>0.67 b</td>
<td>4.92 b</td>
</tr>
<tr>
<td>Open field</td>
<td>6.73 a</td>
<td>5.92 a</td>
<td>29.38 a</td>
</tr>
<tr>
<td>P Value</td>
<td>0.0006</td>
<td>0.0019</td>
<td>0.0034</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mulch</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare ground</td>
<td>3.41</td>
<td>1.88</td>
<td>9.25</td>
</tr>
<tr>
<td>Black polyethylene</td>
<td>3.79</td>
<td>5.88</td>
<td>21.63</td>
</tr>
<tr>
<td>BioAgri</td>
<td>3.38</td>
<td>3.25</td>
<td>24.00</td>
</tr>
<tr>
<td>BioTelo</td>
<td>3.13</td>
<td>2.50</td>
<td>20.88</td>
</tr>
<tr>
<td>SB-PLA</td>
<td>3.28</td>
<td>5.13</td>
<td>17.25</td>
</tr>
<tr>
<td>Weed Guard</td>
<td>3.21</td>
<td>1.13</td>
<td>9.88</td>
</tr>
<tr>
<td>P Value</td>
<td>0.9713</td>
<td>0.4759</td>
<td>0.1078</td>
</tr>
</tbody>
</table>

a High tunnels (‘Solo’ model, Haygrove LTD, UK) and open field plots (37 m long x 8 m wide x 3 m high) were replicated four times and each year trials occurred from mid-May to early-October.

b Mean values within columns followed by the same letter in a column are not significantly different (P = 0.05) as determined by LSD.

c Values presented are means back transformed from rank transformations.

x A lesion indicates one portion of an infected plant (leaf, stem, flower and/or fruit) affected during weekly field ratings; means calculated from four field plot replications with seven plants in each replicated mulch treatment.

y Mulches include commercially available biodegradable mulches BioAgri, 0.02 mm (BA; BioBag, Palm Harbor, FL) and BioTelo, 0.02 mm (Dubois Agrinovation, Waterford, ON, CAN); one cellulose mulch control, WeedGuardPlus, 0.23 mm (Sunshine Paper Co. LLC, Aurora, CO); one standard plastic mulch control, black polyethylene, 0.03 mm (Berry Plastics Corporation, Evansville, IN); and, one bare ground control.

z Experimental spunbond poly(lactic) acid (SB-PLA) mulch was modified annually: 2010 colored white, 0.64 mm, 2011 colored black, 0.58 mm, and 2012 colored black, 0.39 mm and a 75:25 mix of PLA:PHA; PLA = poly(lactic)acid and PHA = polyhydroxyalkanoates (Nature Works LLC, Blair, NE).
TABLE 4. Relative area under disease progress curve (RAUDPC) values for physiological leaf roll on tomato as affected by five agricultural mulches and bare ground in experimental field trials comparing high tunnel and open field production systems near Mount Vernon, WA in 2010, 2011 and 2012.

<table>
<thead>
<tr>
<th>Location</th>
<th>2010&lt;sup&gt;y&lt;/sup&gt;</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>High tunnel</td>
<td>---</td>
<td>21.4 a</td>
<td>30.8</td>
</tr>
<tr>
<td>Open field</td>
<td>---</td>
<td>15.3 b</td>
<td>30.5</td>
</tr>
<tr>
<td>P Value</td>
<td>---</td>
<td>0.0178</td>
<td>0.6730</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mulch</th>
<th>High tunnel</th>
<th>Open field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare ground</td>
<td>42.8</td>
<td>11.9 c</td>
</tr>
<tr>
<td>Black plastic</td>
<td>48.6</td>
<td>52.3 a</td>
</tr>
<tr>
<td>BioAgri</td>
<td>52.7</td>
<td>47.7 a</td>
</tr>
<tr>
<td>BioTelo</td>
<td>49.6</td>
<td>49.0 a</td>
</tr>
<tr>
<td>SB-PLA&lt;sup&gt;z&lt;/sup&gt;</td>
<td>45.2</td>
<td>15.5 c</td>
</tr>
<tr>
<td>WeedGuardPlus</td>
<td>50.2</td>
<td>37.6 b</td>
</tr>
<tr>
<td>P Value</td>
<td>0.6556</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>x</sup> In 2010, a significant interaction was detected between mulch treatment and location and therefore data were analyzed by location using PROC GLM (SAS v. 9.2; SAS Institute Inc., Cary, NC).

<sup>y</sup> High tunnels (‘Solo’ model, Haygrove LTD, UK) and open field plots (37 m long x 8 m wide x 3 m high) were replicated four times.

<sup>v</sup> Mean values within columns followed by the same letter in a column are not significantly different (P = 0.05) as determined by Fisher’s protected LSD.

<sup>W</sup> RAUDPC calculated by dividing AUDPC by range of ratings: in 2010 physiological leaf roll (PLR) was observed for 48 days in both production systems, in 2011 PLR was observed for 96 days in high tunnels and 76 days in open field plots, in 2012 PLR was observed for 77 days in both high tunnel and open field systems.

<sup>z</sup> Mulches include commercially available biodegradable mulches BioAgri, 0.02 mm (BioBag, Palm Harbor, FL) and BioTelo, 0.02 mm (Dubois Agrinovation, Waterford, ON, CAN); one cellulose mulch, WeedGuardPlus, 0.23 mm (Sunshine Paper Co. LLC, Aurora, CO); and, one standard plastic mulch control, black polyethylene, 0.03 mm (Berry Plastics Corporation, Evansville, IN) and each mulch treatment was replicated four times each in both production systems with seven tomato plants per plot.

<sup>W</sup> Experimental spunbond poly(lactic) acid (SB-PLA) mulch was modified annually: 2010 colored white, 0.64 mm, 2011 colored black, 0.58 mm, and 2012 colored black, 0.39 mm and a 75:25 mix of PLA:PHA; PLA = poly(lactic)acid and PHA = polyhydroxyalkanoates (NatureWorks LLC, Blair, NE).
TABLE 5. Comparisons of diseased fruit of tomato cultivar Celebrity at harvest for five agricultural mulches and bare ground in experimental field trials on high tunnel and open field production systems near Mount Vernon, WA in 2011 and 2012.

<table>
<thead>
<tr>
<th>Location</th>
<th>Late blight 2011</th>
<th>Late blight 2012</th>
<th>Gray mold 2011</th>
<th>Gray mold 2012</th>
<th>White mold 2011</th>
<th>White mold 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>High tunnel</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Open field</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Number of diseased fruit at harvest**

<table>
<thead>
<tr>
<th>Mulch</th>
<th>Late blight 2011</th>
<th>Late blight 2012</th>
<th>Gray mold 2011</th>
<th>Gray mold 2012</th>
<th>White mold 2011</th>
<th>White mold 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare ground</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Black polyethylene</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BioAgri</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BioTelo</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SB-PLA</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WeedGuardPlus</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*p* Each year the total number of diseased fruit at harvest were tallied from four replications of high tunnel and open field production systems and tomatoes were grouped by mulch treatment (each treatment included in both production systems). In 2011 only 11 fruits total were affected, but one tomato was severely rotted such that multiple fungi and bacteria were isolated and no causal agent could be identified; this category was not included in the table. In 2012 a total of six fruits were affected by disease over the entire trial but as in 2011 one tomato was rotted such that a single causal agent could not be identified.

*p* Mulches include commercially available biodegradable mulches BioAgri, 0.02 mm (BioBag, Palm Harbor, FL) and BioTelo, 0.02 mm (Dubois Agrinovation, Waterford, ON, CAN); one cellulose mulch, WeedGuardPlus, 0.23 mm (Sunshine Paper Co. LLC, Aurora, CO); and, one standard plastic mulch control, black polyethylene, 0.03 mm (Berry Plastics Corporation, Evansville, IN).

*p* Experimental spun bond poly(lactic) acid (SB-PLA) mulch was modified annually: 2010 colored white, 0.64 mm, 2011 colored black, 0.58 mm, and 2012 colored black, 0.39 mm and a 75:25 mix of PLA:PHA; PLA = poly(lactic)acid and PHA = polyhydroxyalkanoates (NatureWorks LLC, Blair, NE).
TABLE 6. Yield comparisons for tomato cultivar Celebrity grown under five agricultural mulches and bare ground in experimental field trials on high tunnel and open field production systems near Mount Vernon, WA in 2010, 2011, and 2012.

<table>
<thead>
<tr>
<th>Location</th>
<th>Total fruit yield&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Total weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number</td>
<td>2010&lt;sup&gt;v&lt;/sup&gt;</td>
</tr>
<tr>
<td>High tunnel</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2010&lt;sup&gt;v&lt;/sup&gt;</td>
<td>2011&lt;sup&gt;v&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bare ground</td>
<td>34.4 c</td>
<td>55.3 ab</td>
</tr>
<tr>
<td>Black plastic</td>
<td>43.1 ab</td>
<td>59.8 a</td>
</tr>
<tr>
<td>BioAgri</td>
<td>51.1 a</td>
<td>56.5 a</td>
</tr>
<tr>
<td>BioTelo</td>
<td>49.6 ab</td>
<td>62.3 a</td>
</tr>
<tr>
<td>SB-PLA&lt;sup&gt;z&lt;/sup&gt;</td>
<td>39.0 bc</td>
<td>47.8 b</td>
</tr>
<tr>
<td>WeedGuardPlus</td>
<td>42.9 abc</td>
<td>46.6 b</td>
</tr>
<tr>
<td>P Value</td>
<td>0.0083</td>
<td>0.0036</td>
</tr>
</tbody>
</table>

<sup>1</sup>Tomato fruit were harvested when at least 75% red. Each treatment (mulch x production system) consisted of seven plants and was replicated four times.

<sup>u</sup>High tunnel (‘Solo’ model, Haygrove LTD, UK) and open field plots were 37 m long x 8 m wide x 3 m high.

<sup>v</sup>Percent incidence values back-transformed from rank transformation for presentation.

<sup>w</sup>Percent incidence values back-transformed from square root transformation for presentation.

<sup>x</sup>Numbers within a column followed by the same letter are not significantly different (P=0.05) as determined by LSD.

<sup>y</sup>Mulches include commercially available biodegradable mulches BioAgri, 0.02 mm (BioBag, Palm Harbor, FL) and BioTelo, 0.02 mm (Dubois Agrinovation, Waterford, ON, CAN); one cellulose mulch, WeedGuardPlus, 0.23 mm (Sunshine Paper Co. LLC, Aurora, CO); and, one standard plastic mulch control, black polyethylene, 0.03 mm (Berry Plastics Corporation, Evansville, IN).

<sup>z</sup>Experimental spunbond poly(lactic) acid (SB-PLA) mulch was modified annually: 2010 colored white, 0.64 mm, 2011 colored black, 0.58 mm, and 2012 colored black, 0.39 mm and a 75:25 mix of PLA:PHA; PLA = poly(lactic)acid and PHA = polyhydroxyalkanoates (Nature Works LLC, Blair, NE).
Fig. 2. Comparison of soil temperatures in non-raised beds covered with five agricultural mulches and a bare ground control used for growing tomato cultivar Celebrity in experimental field trials on high tunnel (HT) and open field (OF) production systems near Mount Vernon, WA. 1A, 2010 HT; 1B 2010 OF; 2A, 2011 HT; 2B, 2011 OF; 3A, 2012 HT; 3B, 2012 OF.
High tunnel

Transplanted May 12
Recorded until October 28

Open field

Transplanted May 31
Recorded until October 19
Due to faulty sensors, soil temperature was not collected in open field plots at 104, 105, 110, 111, 112, and 116 DAT in 2010; or in the open field SB-PLA-10 treatment at 2 to 6, 71 to 76, and 117 DAT in 2010; or in the BioTelo-high tunnel treatment from 20 to 25, 37 to 39, and 70 to 71 DAT at 2011, otherwise sensors collected data from mid-May to early-October.

Average daily soil temperatures measured at 5 cm depth and calculated from 15-minute interval recordings by sensors attached to a Hobo U-30 Weather System (Onset Computer, Bourne, MA), and in the center of each mulch treatment subplot (4 m long x 0.9 m wide) in one high tunnel (37 m long x 8 m wide x 3 m high; ‘Solo’ model, Haygrove LTD, UK) row and one open field row (each 37 m long x 0.9 m wide).

Mulches include commercially available biodegradable mulches BioAgri, 0.02 mm (BA; BioBag, Palm Harbor, FL) and BioTelo, 0.02 mm (BT; Dubois Agrinovation, Waterford, ON, CAN); an experimental spunbond poly(lactic) acid (SB-PLA) mulch was modified annually: 2010 colored white, 0.64 mm, 2011 colored black, 0.58 mm, and 2012 colored black, 0.39 mm and a 75:25 mix of PLA:PHA; PLA = poly(lactic) acid and PHA = polyhydroxalkanoates (NatureWorks LLC, Blair, NE); one cellulose mulch, WeedGuardPlus, 0.23 mm (WG; Sunshine Paper Co. LLC, Aurora, CO); one standard plastic mulch control; black polyethylene, 0.03 mm (BP; Berry Plastics Corporation, Evansville, IN) as a standard mulch; and, one bare ground control (BG).
Fig. 3. Air temperature measured in the center of one high tunnel (HT) and one open field (OF) main plot for tomato cultivar Celebrity grown near Mount Vernon, WA, 2010, 2011, and 2012.

Air temperature measured in 15-minute intervals recorded by sensors attached to a Hobo U-30 Weather System (Onset Computer, Bourne, MA) placed in the center of a high tunnel (37 m long x 8 m wide x 3 m high; ‘Solo’ model, Haygrove LTD, UK) and open field main plot (37 m long x 0.9 m wide). Seedlings were transplanted into high tunnel plots prior to open field plots.
Fig 4. Severity of late blight on tomato cultivar Celebrity grown under high tunnel and open field production systems in a field experiment near Mount Vernon, WA, 2010. **A**, late blight severity (1% of plot affected) of tomato plants grown under a high tunnel compared to **B**, severe late blight (80% of plot affected) in an open field plot.
Fig. 5. Rating scale used to assess differences in the severity of physiological leaf roll (PLR) on tomato among five agricultural mulches and a bare ground control in an experiment comparing high tunnel and open field production near Mount Vernon, WA, 2010, 2011, and 2012.
Fig. 6. Tomato pith necrosis as observed in an experimental field trial near Mount Vernon, WA in 2011; all samples were removed from open field plots after final fruit harvest. A, discolored pith tissue and dry rot creating “ladder” effect; B, advanced stage where whole pith is rotted leaving hollow sections compared to C, a non-affected plant with green solid pith tissues and symptoms observed during field ratings included D, dark streaks forming acropetally along stem and petioles E, final leaf stages showing small chlorotic areas, and necrotic lesions extending from leaf margin to green midvein, F, adventitious roots breaking through stem tissues and forming along stem starting at mid-canopy, and G, tomato showing a ring of discoloration at blossom end.
Fig. 7. Bacterial stem rot suspect on tomato cultivar Celebrity in an open field experimental plot laid with BioTelo mulch near Mount Vernon, WA in 2012. A, whole plant wilted within six days; B, hollow stem noted at site of wound caused during pruning; C, reddish-brown pith tissue from the upper canopy section; D, pitting around colonies of isolate Pbc.2012-5 on Hildebrand’s C medium (left) at 24 hours and (right) 13 days; E, oxidase test for isolates Pbc.2012-1 (left) as the negative control, Pbc.2012-3 (middle) and Pbc.2012-5 (left).
Fig. 8. Suspected early blight on leaves of tomato cultivar Celebrity grown with BioAgri (BioBag, Palm Harbor, FL) and black polyethylene (Berry Plastics Corporation, Evansville, IN) agricultural mulches in a study comparing high tunnel and open field production systems near Mount, Vernon WA in 2011.
Fig. 9. Disease symptoms on fruit harvested from experimental field trials evaluating five agricultural mulches in high tunnel and open field production systems near Mount Vernon, WA, 2011 and 2012. A, late blight affected tomato with bronze skin discoloration localized to surface tissues; B, gray mold affected fruit, sporulation visible on the whole fruit and inner tomato tissues rotted; C, tomato with white mold, white mycelia at blossom end and sclerotia forming in rotted tissues.
CHAPTER THREE

COMPARISON OF DISEASE OUTBREAKS ON LETTUCE CULTIVARS GROWN ORGANICALLY WITH POLYETHYLENE MULCH IN HIGH TUNNEL VS. OPEN FIELD SETTINGS IN WESTERN WASHINGTON

Abstract

Diseases affecting lettuce (Lactuca sativa L.) in high tunnel and open field production in western Washington (WWA) were compared in consecutive experimental field trials planted April to June during the 2010, 2011 and 2012 growing seasons. Cultivars of four lettuce types (Boston, Leaf, and Romaine) were represented and all plots were managed organically. Gray mold (Botrytis cinerea), lettuce drop (Sclerotinia sclerotiorum) and Verticillium wilt (Verticillium tricorpus) were observed each year in both production systems. The incidence of gray mold incidence was higher in high tunnel than open field plots in 2010, and significantly (P = 0.004) higher in 2011. Compared to other cultivars, Coastal Star and Jericho, both Romaine types, had significantly (P = 0.0001) higher gray mold incidence in 2010, and Green Star, a leaf type, developed significantly (P = 0.0001 and P = 0.007) less gray mold than other tested cultivars in 2010 and 2011, respectively. In 2012, gray mold incidence was significantly (P = 0.0004) higher for cv. Coastal Star produced in high tunnel plots, and significantly (P = 0.0001) higher for cv. Red Sails grown in the open field plots. Lettuce drop was observed all three years, but no significant differences were observed by production system or cultivar in 2010 or 2011. However, incidence of lettuce drop was significantly (P < 0.0001) higher in high tunnel versus open field plots in 2012. Verticillium wilt incidence was not significantly affected
by production system in any year but incidence increased annually from 0.2% to 14.4%. Three isolates of *V. tricorpus* obtained from lettuce plants in 2010 and 2011 proved pathogenic on lettuce in repeated greenhouse inoculation trials. Disease severity ratings per infected lettuce head at time of field harvest ranged from 1 to 100% and 0.1 to 100% for gray mold, 5 to 100% and 1 to 100% for lettuce drop, and 1 to 50% and 1 to 5% for Verticillium wilt in 2011 and 2012, respectively. Green Star proved an ideal cultivar for WWA as this leaf type had high yields, showed some resistance to gray mold, and was not seriously affected by lettuce drop or Verticillium wilt. However, incidence and severity for all observed diseases did not translate into loss of total yield for either production system.

**Introduction**

Lettuce (*Lactuca sativia* L.), a high value, cool weather crop, is produced in Washington as a fresh market and seed crop, making the production of disease-free lettuce plants and seed critically important for growers (31; 99). As of 2010, fresh market lettuce in western Washington was grown primarily in Pierce, Thurston, King, Snohomish and Skagit Counties on 261 acres whereas lettuce as a seed crop was grown in western Washington in Jefferson County and in eastern Washington in Grant and Adams Counties on less than 50 acres (2). Small-scale lettuce production (less than 10 acres) is common in western Washington (2) but there is high demand for locally grown lettuce in the region (3), which includes lettuce that is grown organically. The latest lettuce yield data recorded for Washington by the National Agriculture Statistics Service was in 1999, which listed 210 centum weight (approximately 9,525 kg) per acre (79).

In western Washington lettuce for fresh market production is grown primarily during the spring (April to June) in western Washington when conditions are often cool and wet. Average
air temperature and precipitation in Skagit County during these months are 12°C and 16.6 cm (46), respectively. Spring environments can favor many lettuce foliar and root rot pathogens that cause crop losses (6). Tip burn, caused by calcium deficiency, reduces lettuce quality and can affect marketability if tip burn occurs on leaves within the lettuce head.

The common foliar pathogens on lettuce in the Pacific Northwest include *Bremia lactucae* Regal, a downy mildew (Pscheidt 2009), which infects lettuce leaves in the presence of free moisture or approximately 100% relative humidity, and can cause severe crop loss in Washington when temperatures are between 6 and 12°C (31; 60). Plants with downy mildew develop yellow lesions between veins where the pathogen invades host tissue, and later the lesions turn necrotic. Sporangiospores are produced on the underside of leaves and give the lesions a felt-like appearance; these are dislodged and dispersed by wind. *Botrytis cinerea* Pers. ex Fr. (teleomorph *Botryotinia fuckeliana* Whetzel) causes another serious foliar disease called gray mold, and infects wounded tissues and senescing leaves when free moisture is present or humidity reaches 90% and temperatures are between 15 and 25°C (5). Infected leaves develop brown lesions. Lettuce heads become chlorotic and wilted when infection occurs at the soil line. Abundant gray, powdery conidia are produced which are dispersed by wind; occasionally, sclerotia form in decaying tissues. Anthracnose is a foliar disease on lettuce caused by *Microdochium panattonianum* (Berl.) Sutton, Galea & Price (= *Marssonina panattoniana* (Berl.). Magn.), and infects foliage optimally when free water is available and temperatures range from 20 to 22°C (32). Infected leaves develop sunken, water-soaked lesions and in advanced stages the lesions enlarge and the centers become completely rotted, leaving behind holes. Conidia are produced in lesions, and are splash-dispersed by rain and irrigation water (48).
Among the soilborne pathogens commonly affecting lettuce in the region (Pscheidt 2009) is *Pythium ultimum* Trow, which infects young lettuce seedlings, causing damping off. Infection of root tissues requires moist, cool soils at or below 15.6°C (28; 31). *S. sclerotiorum*, the cause of lettuce drop, also occurs during wet periods when sclerotia are stimulated to germinate via mycelium (myceliogenic germination) or by forming ascocarps with ascospores (carpogenic germination) (24; 47). Myceliogenic and carpogenic germination rates are highest when soil water potentials are maintained at -0.01 MPa (47) and germination has been reported to occur between 12 to 28°C and 10 to 25°C, respectively (1; 47). Both the mycelia and ascospores released from apothecia and dispersed via wind are capable of penetrating wounded and senescing tissues of various hosts (29; 49). Lettuce drop is characterized by plant collapse preceded by girdling, brown soft rot and prolific white mycelia in crown tissues. Sclerotia develop in the crown and head during advanced stages.

Verticillium wilt of lettuce is caused by *V. dahliae* Kleb. and *V. tricorpus* I. To date, two races of *V. dahliae* have been reported for lettuce, Race 1 and Race 2 (83). Severe crop loss can occur in fall-harvested lettuce in large lettuce production areas such as California, with infections caused by *V. dahliae* and to a lesser extent by *V. tricorpus* (15; 58). In Japan, a virulent strain of *V. tricorpus* was reported to cause Verticillium wilt in lettuce and isolates from Japan proved genetically distinct from U.S. isolates (77). Infection by *V. dahliae* occurs in wet soils when temperatures range from 21 to 27°C (8). Environmental studies regarding infection by *V. tricorpus* are lacking, which might be due to low infection rate and the primarily saprophytic nature of this pathogen. Qin et al. (58) reported that isolates of *V. tricorpus* from lettuce grown in California have optimum growth at 20°C on potato dextrose agar and Korolev and Katan (41) reported optimum growth of isolates from Israel at 24°C on Czapek-Dox agar. Symptoms of
Verticillium wilt are evident in nearly mature lettuce, after heads start to form. The most common symptoms of Verticillium wilt are one-sided chlorosis and wilting of wrapper leaves, v-notched necrosis along leaf margins, discolored vascular tissues, stunting, and eventual plant necrosis and collapse. Microsclerotia are often produced in senescing foliar and root tissues (82).

Methods for controlling these diseases on lettuce include one or more of the following: (i) altering environmental conditions so they are not favorable for pathogen growth; (ii) practicing crop rotation; (iii) applying fungicide or biocontrol products for the purpose of reducing disease by either protecting plant tissues or suppressing pathogen growth through microbial antagonism; and, (iv) planting resistant cultivars (6; 26; 67; 95). For organic production systems in particular, cultural practices can help reduce disease by altering environmental conditions conducive for disease outbreaks. Methods like raising beds to improve soil drainage, widening plant spacing to decrease humidity pockets, and employing drip irrigation and protective covers to reduce leaf wetness are used for lettuce production in some areas of the U.S. (52; 59).

Crop rotation is one cultural practice that can be useful for some plant diseases, but has limited success with pathogens such as *S. sclerotiorum* and *V. dahliae* which persist as sclerotia and microsclerotia in soil for years (6; 55; 63), and are capable of infecting 408 and 191 plant species, respectively (9; 55). One rotation scheme to be avoided is lettuce and strawberry, since both crops are susceptible to both pathogens. The threshold of inoculum required to cause Verticillium wilt in lettuce occurs when microsclerotial densities are >100 per gram of soil (4) even though thresholds for strawberry are only three microsclerotia per gram of soil. Although microsclerotia thresholds have not been determined for Verticillium wilt in lettuce caused by *V. tricorpus*, Taylor (76) reported that *V. tricorpus* populations in soil changed with seasons in New Zealand, increasing in May and June, but declining from July to April.
Corn and wheat are often recommended as rotation crops when *Verticillium* populations are low (22; 48), and these crops may offer some control since they are considered non-hosts for *Verticillium* pathogens. However, Isaac (34) reviewed cases in which grass covers were not effective for the reduction of microsclerotia populations of *V. dahliae*. Crop rotation can also be used to incorporate certain plant residues that may help diminish levels of soil inoculum. In California, using either broccoli or Brussels sprouts in rotation with strawberry reduced viable microsclerotia of soilborne *V. dahliae*, and similar results were obtained from broccoli rotations with cauliflower (75; 93). Broccoli rotations could be a viable option for control of Verticillium wilt in lettuce, especially in organic production where fumigation is prohibited. Shipton (65) reviews the importance of crop rotation by evaluating topics regarding soilborne diseases in monoculture.

Chemical measures can be useful for lettuce disease control but some fungicides may not be allowed in organic production systems or effective in managing fungicide-resistant plant pathogen populations. Repeated protective applications may be necessary due to continuous plant growth, wash off, and UV degradation, all of which can increase the costs of production (11; 67; 94). Biocontrol applications may not be effective in controlling the target pathogen if specific environmental conditions are not met to optimize growth of the antagonist or if inoculum is re-introduced from outside sources, such as spores dispersed from nearby fields in the wind (14; 23).

Resistant and partially resistant cultivars offer protection against some lettuce diseases or some pathogen strains. Strain specific resistance for downy mildew in lettuce is available, but may offer limited disease control in some areas where specific strains prevail (44; 60). According to seed distributors (see Table 1), cultivars Adriana, Ermosa, Green Star, and Nevada
provide some race-specific downy mildew resistance. However, genetic resistance of the host can be overcome due to selection pressure for virulent strains of the pathogen. Currently, there are no known cultivars of lettuce resistant to *B. cinerea*, *S. sclerotiorum*, and *V. dahliae* race 2 (20; 26; 74; 83; 88). There is evidence of partial resistance to *S. sclerotiorum* in some wild (*L. virosa* L.) and cultivated (*L. sativa* L.) lettuce lines, as observed in Arizona field trials, and the resistance was not associated with disease escape from rapid bolting or small head size (26). Greenhouse studies have also shown partial resistance to *S. sclerotiorum* in cultivated and prickly lettuce lines when compared to susceptible cultivars (88). Resistance to *V. dahliae* Race 1 has also been demonstrated in California field and greenhouse trials with various cultivars (27; 83).

One potential method of managing lettuce diseases that has not yet been explored in western Washington is the use of high tunnels. High tunnels are protective structures that trap solar energy, increasing air and soil temperatures while physically blocking rainfall, which reduces leaf wetness and excess soil moisture. Growers in western Washington are interested in utilizing high tunnels for lettuce production to increase lettuce yield and quality (84) and to better optimize temperatures for lettuce production, which generally range from 13 to 18.3°C (31). Increasing air and soil temperatures while reducing leaf wetness and excess soil moisture could be advantageous for spring-grown lettuce in the Pacific Northwest.

Lettuce production has benefited from high tunnel structures in many areas of the U.S. and in Canada due to early production and increased quality (13; 59; 81; 84; 89). In fact, lettuce is the number two crop grown in high tunnels in the U.S. following tomatoes (13; 53). High tunnels provide an attractive alternative to chemical disease control methods for both organic and conventional farmers by reducing leaf wetness, a contributing factor to many plant diseases (30;
There may be drawbacks to high tunnel adoption such as increased costs of production through labor and equipment. Also, accrued heat may affect crop maturity and bitterness, reduce phenolic content (reduces antioxidant potential), increase tip burn when high humidity occurs, and contribute to some diseases (57; 59; 68; 96; 97; 99). A recent crop budget report produced by the Washington State University School of Economics estimated that production costs for one acre of field-grown lettuce in western Washington is approximately $16,117, whereas net returns are estimated to be $22,651 (21).

The objective of this study was to grow organically managed lettuce in high tunnels and open fields in western Washington in order to (i) contrast naturally-occurring disease epidemics in the two types of production systems, and (ii) determine the reaction of selected lettuce cultivars to observed diseases by comparing disease incidence, severity, and yield.

**Materials and Methods**

**High tunnel and raised bed construction.** An experimental field trial was established each spring at Washington State University Mount Vernon Northwestern Washington Research and Extension Center (WSU Mount Vernon NWREC; lat. 48°43’24” N, long. 122°39’09” W, elevation 6m) for three consecutive cropping cycles from 2010 to 2012. The study design was a two by six factorial, and consisted of high tunnel and open field plots and six lettuce cultivars arranged in split plot. The field site had been managed organically with small grain and legume rotations for four years prior to this study. Six lettuce cultivars (Table 7) were seeded in the greenhouse in 72-cell plug trays in Sunshine Natural and Organic Growing Mix 9-3-2 (SunGro Horticulture Distribution Inc., Bellevue, WA) each year, but in 2012, Nevada and Valmaine cultivars were seeded in Sunshine Natural and Organic Growing Mix #3. One objective of this
study was to evaluate lettuce cultivar performance for production in western Washington, so a few cultivars/types were changed during the 2012 growing season. Seedlings were grown for five weeks with a 12-hour photoperiod at 18.3 to 23.8°C. Open-ended high tunnels (‘Solo’ model, Haygrove LTD, UK; 36.6 m long x 8.4 m wide x 3.0 m high) were erected 19 Apr in 2010, 29 Apr in 2011, and 20 Apr in 2012. Raised beds (0.91 m wide x 0.23 m high x 4.3 m long) were formed with a Model 2600 Raised Bed Mulch Layer (RainFlo Irrigation, East Pearl, PA). A single drip tape line (low flow, 1.6 cm diameter, 0.2 mm thick, 20.3 cm emitter spacing; John Deere Water, San Marcos, CA) and black plastic mulch (0.03 mm thick embossed; Berry Plastics Corporation, Evansville, IN), was laid on the beds 7 Apr in 2010, and 20 Apr in 2011 and 2012. Seedlings were foliar fertilized prior to transplanting with Drammatic K (2.0-5.0-0.2) liquid fertilizer (Dramm Corporation, Manitowoc, WI) at 250:1 water:fertilizer ratio, once on 25 Mar 2010 and once on 11 Apr in 2012. In 2011, Organic BioLink 3-3-3 liquid fertilizer (Westbridge Ag Products, Vista, CA) was used twice at 250:1 water:fertilizer ratio, on 25 Mar and 7 Apr. Seedlings were hardened-off for seven days outdoors, and then 28 six-week-old seedlings were transplanted by hand mid- to late-Apr (Table 8) into each high tunnel and open field subplot. Planting was in two staggered rows with spacing of 30.58 cm in-row, and 30.48 cm between rows, and 182.98 cm bed center-to-center (see Chapter One Fig. 1; See Table 8 for irrigation and fertigation schedules).

Environmental monitoring. Environmental data were collected in 15-minute intervals with Hobo U-30 Weather Stations (Onset Computer, Bourne, MA), in an adjacent row within one plot replicate. Environmental data included air temperature, leaf wetness, photosynthetically active radiation (PAR), relative humidity, soil temperature at 5 cm depth, and wind speed, and were recorded from 22 Apr to 1 Jul in 2010, 30 Apr to 27 Jun in 2011 (nine days after
transplanting (DAT)), and 26 Apr to 9 Jul in 2012. In 2010 environmental data were not collected between 28 and 31 May in high tunnels, and soil temperatures were not collected until 19 May (27 DAT) in high tunnels or until 25 May (33 DAT) in open fields, due to an equipment malfunction. In an adjacent bed that received the same irrigation schedule, soil moisture was determined with Watermark irrometers (Irrometer Company Inc, Riverside, CA) installed as described by Shock et al. (66). Soil moisture at 20 cm depth was recorded in the mornings once per week.

**Disease, insect, and weed control.** In 2010 lettuce was planted following four years of cover crops, but in 2011 and 2012 lettuce followed strawberry in rotation. All pesticides used in this study were allowable for certified organic production. In 2010, one application of PyGanic Crop Protection EC 1.4 (MGK Company, Minneapolis, MN) was made on 11 Jun at a rate of 590 g/ha for aphid control, and one application of Entrust Naturalyte Insect Control (Dow Agrosciences LLC, Indianapolis, IN) at 210 g/ha was made to all plots on 18 Jun to control flea beetle. No insecticides were applied in 2011 due to low insect pressure. Prior to bed shaping in 2011, Contans WG (ADVAN, Roswell, GA), a powder containing spores of *Coniothyrium minitans* Campbell, was applied to lettuce rows at a rate of 6.7 kg/ha on 20 Apr. *C. minitans* parasitizes soilborne sclerotia of *S. sclerotiorum*. In 2012, PyGanic EC 5.0 (MGK Company, Minneapolis, MN) was applied on 21 Jun at 1.3 liters/ha to control aphids. All of the above applications were applied with a CP3 back pack sprayer (Cooper Pegler, UK) and a TeeJet flat spray tip (8004VS) nozzle (TeeJet Technologies, Carol Stream, IL). Weeds growing up through planting holes in the polyethylene mulch were removed by hand as needed and weeds growing between beds were removed with a hoe once per week.
Disease outbreak and harvest quality assessments. Disease symptoms were rated weekly for each subplot, 29 Apr to 24 Jun in 2010, 4 May to 24 Jun in 2011, and 17 May to 5 July in 2012. Plants were scored each week as healthy, weak, or dead. Weak plants included those with stunted heads as well as plants that exhibited signs of a disease or symptoms such as chlorosis, necrosis, lesions, or wilt not associated with senescence. Disease incidence was recorded as the number of plants per subplot displaying signs and symptoms of a disease. Disease severity was recorded as percent (0 to 100%) of the subplot affected.

Lettuce subplots were harvested by hand when at least 75% of potentially marketable plants reached the mature head stage or prior to loss of marketability due to disease and disorders, from 15 Jun to 1 Jul in 2010 (54 to 70 DAT), 10 Jun to 27 Jun in 2011 (49 to 66 DAT), and 14 Jun to 9 Jul 2012 (49 to 74 DAT) (Table 9). Mature head stage was determined by assessing firmness of bunched leaves, which was done by hand, or when head diameter reached 30 cm. Lettuce heads were harvested by hand in the morning to minimize wilting, and heads were cut at the soil line and collected one subplot at a time. Total head number and weight per subplot were recorded. Harvested heads were visually inspected for disease and quality. Wrapper leaves were removed, the stem was trimmed to obtain final marketable head weight. Lettuce was deemed marketable if the trimmed head diameter was at least 8 cm and the leaves were not affected by slug damage or tip burn.

Heads that were diseased, noted by crown or head rot, sporulation, and/or stunting were visually rated for disease incidence on a subplot basis, and as percent severity per plant. Severity ratings were recorded with a 1 to 100% scale, in 1% increments from 1 to 5%, 5% increments from 5 to 20%, and then 10% increments from 20 to 100% (Figures 10 through 12). Marketable plants with only a small lesion or plants with up to a few symptomatic leaves were given severity
ratings ranging from 0.1 to 5% depending on degree of wilt, chlorosis, rot, and vascular
discoloration. If a single leaf showed chlorosis and wilt, severity was rated as 0.1% but if the leaf
showed lesions with chlorosis and wilt or if rot was present on an outer leaf, severity was rated
as 1%. At 50% severity, one-half of the crown or head was rotted. Plants that had completely
rotted such that only dry leaves remained were given a severity rating of 100%. In addition to
severity, individual disease symptoms were recorded on a 1 to 4 scale, 1 = 1 to 25%, 2 = 26 to
50%, and 3 = 51 to 75%, and 4 = 76 to 100% affected. This scale was applied to gray mold
ratings for rot, sporulation, and brown midveins, and to lettuce drop for rot, sporulation, and
sclerotia formation. Disease severity was not rated for all subplots in 2010, but was rated in 2011
and 2012.

In 2011 and 2012 heads with tip burn, a calcium deficiency, were counted at harvest, and
weighed separately. Tip burn was not rated using a disease severity scale but visual observations
were made to determine if tip burn was present on inner leaves of lettuce heads, rendering the
head unmarketable (Fig. 13).

**Isolation and identification of pathogens.** Symptomatic tissues obtained from harvested
lettuce heads were used to recover isolates of the observed pathogens *Botrytis, Sclerotinia,* and
*Verticillium.* Pathogens were isolated one of three ways: (i) from surface sterilized plant tissue
that was dipped in 70% EtOH for 30 s, then dipped in 10% NaOCl for 30 s, and finally rinsed
with sterilized deionized water and plated onto various culture media, including half-strength
potato dextrose (½ PDA), NP-10 (10; 36; 69), or water agar), or placed in a moist chamber with
sterile water; (ii) from plant tissue cut with a sterile knife and placed in a moist chamber with 1%
NaOCl; or, (iii) from sclerotia removed from plant tissue with sterile forceps, rinsed with water,
dipped in 70% EtOH for 2 s, passed through a flame, and plated onto ½ PDA. Tissue for
pathogen isolation was removed from representative heads that showed disease symptoms. Colony morphology was observed after incubating cultures for two weeks at 20°C under ambient light conditions.

In 2012, the number, size, and viability of sclerotia formed by *S. sclerotiorum* embedded in lettuce tissue were also tracked based on observations in 2011 that there might be differences by cultivar. Sclerotia were removed from diseased lettuce heads with sterile forceps, placed onto 3 mm x 3 mm mesh sieve, and then placed under running tap water to remove remnant plant tissue. Sclerotia were air dried for 24 h on a laboratory bench under ambient light at 21°C and then stored in a sealed box. At five weeks, sclerotia were weighed and five randomly selected sclerotia were measured with a caliper and plated onto ½ PDA with bromophenol blue (½ PDA + BPB) as reported by Steadman *et al.* (73) except that antibiotics were omitted in order to ascertain viability. The remaining sclerotia were separated into three size classes with sieves: U.S. No. 25 (small; up to 710 µm), U.S. No. 12 (intermediate; 711 µm to 1.70 mm), and US No. 6 (large; 1.71 mm to 3.35 mm) (17).

Following morphological analysis for tentative identification, selected fungal isolates from 2010 and 2011 were sent to WSU Puyallup Research and Extension Center for phylogenetic analysis via direct PCR amplification of the internal transcribed spacer region of the 18S ribosomal RNA gene, using primers ITS4 and ITS6 as described by Calmin *et al.* (12) for Oomycetes, with the following modifications: bovine serum albumin was added to reduce inhibitors as shown by Kreader (43); initial DNA denaturing was limited to 10 min at 94°C; and, PCR products were purified with ExoSAP-IT (Affymetrix Inc., Santa Clara, CA) according to the manufacturer’s directions. DNA was sequenced with Applied Biosystems 3730xl capillary electrophoresis and sequence files annotated with FinchTV software (FinchTV 1.4.0; Geospiza,
Inc., Seattle, WA). Sequence results were used to identify isolates to species with a nucleotide BLAST search. In 2012, *B. cinerea* and *Verticillium* spp. isolates were identified morphologically (34; 35) and *S. sclerotiorum* isolates were identified on ½ PDA + BPB (56; 73) without antibiotics.

**Pathogenicity tests.** Isolates of *B. cinerea*, *S. sclerotiorum*, and *V. tricorpus* from field lettuce heads were tested in 2010 and 2011 in separate greenhouse pathogenicity trials. Conidial suspensions used for inoculation were prepared from one-to-two week-old cultures while 1 cm² agar plugs with uniformly distributed microsclerotia, or mature sclerotia were removed from three-week-old cultures growing on half-strength PDA. Four-week-old lettuce seedlings were inoculated with conidial suspensions, two agar plugs of microsclerotia, or sclerotia, and transplanted into Sunshine Mix #1 (SunGro Horticulture Distribution Inc., Bellevue, WA).

*B. cinerea* isolate Bc.Ls.2010 from the 2010 lettuce harvest was tested on eight Batavia seedlings; the cultivar was selected for its plant architecture that favors water collection at the crown. Six-week-old seedlings were transplanted, watered, and then 1 ml of a 1.0 x 10⁶ suspension of *B. cinerea* conidia was applied to lettuce leaves so that the suspension ran down the midrib and collected in the crown. Immediately after seedlings were inoculated with *B. cinerea*, plants were placed inside large plastic bags, sealed, shaded with Remay cloth, and incubated for 24 hours to achieve high humidity. Additionally, 12 Batavia seedlings were inoculated with sclerotia of *Botryotinia fuckeliana* (Bf.Ls.2010). These six-week-old seedlings were gently wounded by dragging a sterile scalpel 1 cm down the plant crown and then placing 0.05g of sclerotia in the planting hole adjacent to the wound in the planting hole. The *B. cinerea* isolate from the 2011 lettuce harvest, Bc.Ls.2011, was also tested for pathogenicity on five seedlings of Jericho. The suspension of Bc.Ls.2011 was reduced to 1.0 x 10³ conidia per ml
because the Bc.Ls.2010 inoculum density had appeared to be too high due to abundant and rapid sporulation, and there was risk of contaminating nearby plants. Thus, plants inoculated with Bc.Ls.2011 were kept in a separate greenhouse to reduce the potential of cross contamination. Control plants were either inoculated as above with sterile water or wounded but no sclerotia were placed the in planting holes. Plants were harvested at seven and nine weeks post inoculation in the pathogenicity tests on isolates Bc.Ls.2010 and Bc.Ls.2011, respectively.

Twelve seedlings each of Coastal Star and Salinas were used to test pathogenicity of S. sclerotiorum isolate Ss.Ls.2010 acquired from the 2010 lettuce harvest. Lettuce stems were wounded near the crown and sclerotia transferred with sterile forceps into the planting hole, adjacent to the plant wound as described above. Eight Jericho seedlings were inoculated with the 2011 lettuce harvest isolate Ss.Ls.2011 of S. sclerotiorum. Control plants were wounded as above but no sclerotia were placed in planting holes. Plants were harvested at eight and nine weeks post inoculation for pathogenicity tests on isolate Ss.Ls.2010 and Ss.Ls.2011, respectively.

Twelve seedlings each of cultivars Batavia, Coastal Star, Little Gem, Merlot and Salinas were inoculated with the 2010 V. tricorpus isolate Vt.Ls.2010. In this first pathogenicity test, all plants were inoculated twice, first with two agar plugs placed below root tips during transplanting. Five weeks after agar plug inoculation, plants were removed from pots. Roots were rinsed with tap water and sterile scissors were used to remove approximately 1 cm of the roots. Cut root tips were then dipped into a suspension of 2.0 x 10^6 conidia per ml for 5 s, and seedlings were transplanted. Eight seedlings each of Romaine cultivars Coastal Star and Jericho were inoculated in a second pathogenicity test with three V. tricorpus isolates of Vt.Ls.2010 plus two additional isolates from the 2011 lettuce harvests, Vt.Ls.2011-1 and Vt.Ls.2011-2. Lettuce
roots were cut with sterile scissors, and dipped into a conidial suspension as described above. In a third pathogenicity test, two inoculation techniques with the same three *V. tricorpus* isolates, Vt.Ls.2010, Vt.Ls.2011-01, and Vt.Ls.2011-02, were used on five seedlings of Coastal Star. Five plants were inoculated with a conidial suspension as described above but the concentration was increased to $5.0 \times 10^6$ conidia per ml. An additional five plants were inoculated by gently wounding the stem at the soil line with a sterile scalpel, then placing two agar plugs adjacent to the wound in the planting hole. For each of the three pathogenicity tests with *V. tricorpus*, the same lettuce cultivar was also inoculated with either sterile water or water agar plugs, and with *V. dahliae* as a control isolate since *V. dahliae* is a known aggressive pathogen of lettuce (4). The isolate of *V. dahliae*, Vd.Fs.2010, was obtained in 2010 from a diseased strawberry located near lettuce in the high tunnel field experiment. Disease incidence and severity comparisons were made between *V. tricorpus*, *V. dahliae*, and water controls. Plants were harvested at eight, nine, and eight weeks post inoculation for pathogenicity tests one, two, and three, respectively.

Plants were grown on a greenhouse bench at 45.5% relative humidity, 12 hour photoperiod, and 17.7°C average daily temperature in the first pathogenicity test of the 2010 lettuce harvest isolates. For the second and third pathogenicity tests, plants were grown on a greenhouse bench with a 16 hour photoperiod at 21°C.

**Data analyses.** Data regarding disease incidence at harvest were subjected to analysis of variance using the PROC MIXED procedure of SAS v. 9.2 (SAS Institute Inc., Cary, NC) with the Satterthwaite method (62) for assessing degrees of freedom and Least Squared means to obtain mean separations. Either rank or reciprocal transformations were selected using the range method described by Kirk (38) for cases where the data did not fit assumptions of normality and homogeneity. When significant interactions between cultivar and production systems were
detected, PROC GLM was used to obtain means, and mean separations were determined by Fisher’s Least Significant Difference with alpha equal to 0.05 (54). Total lettuce yield and tip burn were subjected to analysis of variance using the PROC MIXED procedure as above except that the Kenward-Roger (37) method was used to determine denominator degrees of freedom for F-tests. Where transformed data failed to meet the assumptions of normality or homogeneity, the data were analyzed non-parametrically after transformation.

Results

Environmental monitoring. Across years, average air temperatures for both production systems were slightly higher each season such that the highest average air temperature was recorded in 2012 and the lowest in 2010 (Fig. 14a and b). In 2010, air temperatures inside the high tunnels were occasionally cooler than temperatures recorded in open fields but these events occurred infrequently and early in the growing season from 3 to 22 May (11 to 30 DAT), and represented only a 0.07 to 1.9°C difference. The lowest temperature, 6.4°C, was recorded on 5 May (13 DAT). Air temperature in 2010 did not exceed 15°C in open field plots until 22 Jun (61 DAT), whereas air temperature inside the high tunnels periodically rose above 15°C for 15 days from 20 May to 30 Jun (28 to 69 DAT) and then ranged from 15.2 to 20.0°C.

In 2011, air temperatures were nearly always higher in high tunnels than in open fields, and reached above 15°C on 3 and 5 Jun (42 and 44 DAT) in both the high tunnels and open field plots, respectively. Air temperatures never reached 20°C for either production system in 2011 but exceeded 15°C for 17 days inside the high tunnels (15.1 to 19.4°C), but only for six days in open field plots (15.9 to 17.6°C). Air temperatures that were lower in the high tunnel relative to the open field were only recorded once on 10 May (18 DAT).
In 2012, air temperatures inside the high tunnels frequently rose above 15°C, beginning 6 May (18 DAT; Fig. 14c); these early season warm temperatures did not occur in 2010 or 2011. Air temperatures were greater than 15°C for 22 and 14 days in the high tunnel and open field plots, respectively; and, air temperatures recorded in the high tunnels remained 0.20 to 2.3°C greater than in the open field.

Precipitation during the 2010 and 2012 growing seasons was similar with 18.2 and 18.4 cm recorded each year, respectively; only 15.3 cm precipitation was recorded in 2011 (Fig. 15A). Leaf wetness reported as accumulative hours of wetness throughout each growing season, was consistently higher in the open field than in the high tunnel plots each year (Fig. 15B), as expected, since open field plots received rainfall and high tunnel plots did not. Lettuce produced in open field plots was exposed to 133, 64, and 281 additional hours of leaf wetness compared to lettuce in high tunnel plots in 2010, 2011, and 2012, respectively. In spite of the small differences in precipitation in 2010 and 2012, leaf wetness in the open field was 11% higher in 2012 compared to 2010. Although precipitation during the 2011 growing season was lowest, the duration of leaf wetness in high tunnel production in 2011 exceeded the 2010 record by approximately 55 hours.

Photosynthetically active radiation (PAR) was reduced in high tunnel production systems compared to open fields in all three years (Fig. 16). Daily PAR was reduced on average by approximately 32, 85, and 101 µE in high tunnels compared to open field plots in 2010, 2011, and 2012, respectively.

Relative humidity ranged between 67 to 97%, and was similar for both production systems across all three years (Fig. 17). Each year, in late-June, towards the end of the growing season when air temperatures were highest, relative humidity in the open field ranged from 0.02
to 10.1%, 0.12 to 4.3%, and 0.03 to 6.9% higher than was recorded in the high tunnel in 2010, 2011, and 2012, respectively. The total number of days in which relative humidity reached or exceeded 90% in open field production was 5, 5, and 10 days compared to 2, 2, and 6 days in high tunnel plots in 2010, 2011, and 2012, respectively.

The field experiment was conducted on mulched beds of Skagit silt loam with drip irrigation. This soil type is considered to be near field capacity when soil moisture tension is approximately 20 to 25 cbar (-0.02 to -0.025 MPa) and dry when the tension is approximately 65 cbar (-0.065 MPa) (72). Soil moisture varied among replications within the high tunnel and open field production systems throughout the growing seasons all three years. Early in each growing season, from 3 to 16 May (11 to 20 DAT), soil moisture remained near saturation. Midway through each growing season 20 May to 13 Jun (28 to 48 DAT), soil moisture tension exceeded 70 cbar (-0.070 MPa) in both production systems. The maximum soil moisture tension in open field plots was 117 cbar (-0.12 MPa) whereas the maximum soil moisture tension was 100 cbar (-0.10 MPa) in high tunnel plots.

Soil temperature at 5 cm depth was generally higher in high tunnel than open field plots in 2010, 2011, and 2012 (Fig. 18). Soil temperature in the high tunnels ranged from 9.5 to 25.3°C in 2010, 14.0 to 21.5°C in 2011, and 9.11 to 23.2°C in 2012. Soil temperature in the open field ranged from 9.1 to 21.7°C in 2010, 13.8 to 20.3°C in 2011, and 8.5 to 22.1°C in 2012. On average, soil temperatures were 2.4, 0.7 and 0.8°C higher in high tunnel versus open field systems in 2010, 2011, and 2012, respectively. Each of the three years, soil temperature within the high tunnel would occasionally drop below the soil temperature in the open field, despite higher air temperatures in the high tunnel. The decline in soil temperature in the high tunnels
only occurred one day in 2010 but occurred for four days from 29 May to 1 Jun (37 to 40 DAT) in 2011, and 6 days from 2 to 6 May (44 to 49 DAT) in 2012.

Wind speed was reduced inside tunnels all three years (Fig. 19). Wind speed, which is a factor in the dispersal of conidia and ascospores was reduced in high tunnels during the 2010 through 2012 growing seasons by 1.6, 1.0, and 0.9 mph, respectively, as compared to the open field.

**Disease outbreaks.** Gray mold, lettuce drop, and Verticillium wilt were observed in each year in both high tunnel and open field plots. Anthracnose, downy mildew, and *Pythium ultimum* were not observed in any year.

**Gray mold.** In 2010 and 2011, gray mold onset was observed in high tunnel and open field plots on 10 and 9 June (49 and 48 DAT), respectively (Table 9). In 2012, gray mold was observed in high tunnels on 24 May (28 DAT) whereas symptoms in the open field were observed two weeks later, on 7 Jun (42 DAT). In both production systems in 2010, sporulation by *Botrytis* was observed on the inner leaves of maturing lettuce heads during weekly field ratings (Fig. 10c), but in 2011 and 2012, sporulation was mostly confined to crown tissues and was not visible until the plant started to wilt. When infection was confined to crown tissues, lettuce plants wilted so quickly that the heads retained green pigment even after plant collapse. Brown, watery midveins also developed in the outer leaves of plants having crown infections. The brown midvein symptom was not observed for gray mold infections initiated within lettuce heads, however. All three years, disease occurred on plants randomly in each plot.

Gray mold occurrences differed each year based on production system, cultivar, and environment. For example, gray mold did not differ by production system in 2010, however, gray mold was significantly (*P* = 0.0037) higher in high tunnels than open field plots in 2011.
(Table 10). In 2012 there was a significant interaction between cultivar and production system for gray mold incidence. Coastal Star and Jericho, both Romaine types, had significantly ($P < 0.0001$) more gray mold than the two cultivars each of leaf and Boston types in 2010. At harvest in 2010, a total of 94 out of 1,299 plants (7%) showed symptoms of gray mold. Sixty six of the 94 affected plants (70.2%) were Jericho, 26 out of 94 plants (28.6%) were Coastal Star, and one each (1%) were Boston types Adriana and Ermosa. Green Star, a leaf type, showed no incidence of gray mold at harvest in 2010. In 2011, Green Star was the only cultivar which showed some gray mold resistance. Green Star was affected by gray mold during the growing season; however, at harvest the heads were not affected. Severity of gray mold at harvest in 2011 was highest for Coastal Star (1 to 100%) followed by Jericho (20 to 50%), then Adriana (10 to 50%), then Ermosa and New Red Fire (both 1 to 50%), and then Green Star (0%). Also in 2011, gray mold severity was consistently higher in Adriana heads harvested from open field compared to heads harvested from high tunnel plots. In 2012, gray mold incidence was significantly ($P = 0.0004$) higher for Coastal Star in high tunnels than in open field plots, but conversely, incidence for Red Sails was significantly ($P = 0.0001$) higher in the open field than in the high tunnel plots. Interestingly, gray mold was noted on only high tunnel harvested heads of Nevada and Valmaine. Gray mold severity was highest in Coastal Star, Valmaine, and Nevada (all 1 to 100%), then Red Sails (1 to 80%), then Concept (1 to 50%), and then New Red Fire (1 to 30%).

Additionally, ratings of individual gray mold symptoms (0 to 4 scale) revealed differences in gray mold severity by production system and cultivar. One symptom observed in 2010 and 2011 was the browning of midveins on outer leaves. In both years, the midvein symptom severity ranged from 1 to 3. In 2011, Adriana, Coastal Star, and Jericho were the only cultivars that had brown midvein symptoms; however, differences were observed in severity
between production systems. Severity ratings of leaves affected by brown midveins in 2011 were higher in open fields (severity = 2 to 3) compared to high tunnels (severity = 1 to 2) for cultivars Adriana and Coastal Star but for Jericho brown midvein symptoms were only visible on heads harvested from high tunnel plots (severity = 0 to 1). In 2012, all cultivars exhibited brown midvein symptoms but severity was more pronounced in outer leaves of Coastal Star, Nevada, and New Red Fire lettuce harvested from high tunnel plots than from open fields, or the other cultivars. No sclerotia were observed on any infected head any year, although isolates recovered from infected lettuce tissue would sometimes produce sclerotia on ½ PDA.

**Lettuce drop.** In 2010, lettuce drop symptoms were observed in high tunnel and open field plots on 17 Jun, eight days after the time of gray mold onset (Table 9). In 2011, lettuce drop coincided with gray mold onset for open field plots on 9 Jun (48 DAT) but onset in high tunnels was delayed in high tunnels and observed on 16 Jun (55 DAT). Lettuce drop was observed as early as 7 Jun (42 DAT) in both production systems in 2012. Since lettuce drop symptoms typically develop within 9 days at 16 to 27°C and 20 to 26 days when temperatures range from 8 to 11°C (95), environmental data were compared between 2010 and 2011 and 2012 to look for trends. Air temperature and soil temperature and moisture ranged greatly from year to year, and it appeared that infection by *S. sclerotiorum* occurred over a wide range of environmental conditions under the protective covers because: (i) air temperatures averaged 14 and 15°C within 10 days of symptom development in high tunnels in 2010 and 2011, respectively, and averages did not change going back 15 days prior to infection; (ii) average soil temperatures recorded in the high tunnels differed all years and ranged from 17 to 20°C; and, (iii) soil moisture tension recorded approximately 10 days prior to lettuce drop symptom development within the high
tunnels ranged from 20 to 73 cbar (-0.02 and -0.073 MPa) in 2010 and from 2 to 34 cbar (-0.002 to -0.034 MPa) in 2011.

Lettuce drop typically affected clusters of plants, regardless of production system, cultivar or year. Disease symptoms and/or signs were noted on a single plant or on two-to-three adjacent plants within a cultivar row or between subplots. Heads harvested in mid-June did not develop thick white mycelial mats typical of lettuce drop, but did develop soft rot symptoms in the core. Heads harvested in late June were severely infected, and both white mycelium and large black sclerotia (>1 cm in length) were commonly found inside lettuce head tissues and near lettuce crowns (Fig. 11D and F). Although the onset of lettuce drop in 2010 and 2011 occurred in early- to mid-June (48 to 57 DAT) in both production systems, diseased lettuce heads only produced sclerotia in late June, 6 to 11 days after the time of the first harvest or 60 to 65 DAT (Table 9).

In 2012, sclerotia were removed from infected heads, dried for five weeks, and then weighed, sorted into size categories, and plated onto media to determine viability. Romaine cultivars Coastal Star and Valmaine harvested 28 Jun to 5 Jul (63 to 70 DAT) in the high tunnel production system produced the highest number of sclerotia (Table 11). The most commonly recovered sclerotial size was intermediate (711 µm to 1.7 mm) for all affected plants.

Lettuce drop incidence did not differ significantly between high tunnel and open field production systems or among lettuce cultivars in either 2010 or 2011, but was significantly ($P < 0.0001$) higher in high tunnel plots in 2012 (Table 10). In 2010, 30 out of 1,299 plants (2%) across all cultivars exhibited symptoms of lettuce drop and 23 out of 30 affected heads (76.6%) came from open fields. In 2011, 38 out of 1,284 (3%) across all cultivars exhibited symptoms of lettuce drop and 18 of these heads (47.4%) came from open fields. In 2012, 75 out of 1,218
heads (6%) across all cultivars were affected by lettuce drop in which 62 out of 75 affected plants (83%) were from high tunnels and 13 out of 75 affected plants (17%) were from the open field. Evaluating incidence in 2012 by cultivar, incidence was highest in Coastal Star and New Red Fire, affecting 24 heads each cultivar out of 75 total affected heads (32%). As with gray mold above, lettuce drop severity between production systems and among cultivars proved to be difficult to analyze due to differences in the stage of infection. In 2012, lettuce drop severity was greatest in heads of Coastal Star from high tunnel plots with sclerotial formation occurring on up to 75% of the plants compared to up to 25% in open field production. Heads of Coastal Star, Concept, Nevada, Red Sails, and Valmaine from high tunnel plots had higher severity ratings for lettuce drop compared to ratings for heads from open field production in 2012.

**Verticillium wilt.** Although Verticillium wilt was observed all three years, disease incidence was not affected by cropping system and no significant differences were observed between the high tunnel and open field plots in any year (Table 10). Verticillium wilt symptoms in the field were mild all three years (Fig. 12), but each year disease incidence increased. Mild symptoms encompassed v-shaped chlorotic patterns on outer leaves, and sometimes wilt, stunting, or vascular discoloration. In 2010, only three diseased heads from high tunnel plots were detected but not until after harvest, when senescing leaves were removed revealing chlorosis and vascular discoloration inside cut crown tissues. Vascular discoloration was localized in crown tissues, never in leaf vascular tissues. Only Coastal Star and Jericho were affected by Verticillium wilt in 2010.

In 2011, on 22 Jun (61 DAT) symptoms of Verticillium wilt were noted in the field, but this was after 46% of the plots had already been harvested. Of the 25 symptomatic plants (2% of total heads harvested) 16 were from the high tunnel and nine were from the open field plots, and
60% affected heads were still marketable. At harvest, significant differences were observed among cultivars (Table 10). The Romaine cultivar Jericho had a significantly ($P < 0.001$) higher incidence of Verticillium wilt whereas the disease was not observed in New Red Fire, a leaf type. Disease severity per infected plant at the time of harvest ranged from 1 to 50% for Adriana, 1 to 5% for Coastal Star and Jericho, 1% for Ermosa, and 5% for Green Star.

In 2012, 171 out of 1,218 plants (14%) exhibited mild symptoms of Verticillium wilt (65 heads from high tunnels and 111 from open field plots) but all heads were still marketable. Approximately 42% of all Coastal Star heads exhibited symptoms of Verticillium wilt and 71% of all symptomatic plants across all cultivars were from the open field plots. Of the affected heads, 54% were Coastal Star, 33% were Concept, 6% Valmaine, 5% New Red Fire, and 3% Nevada. Symptoms of Verticillium wilt were not observed in Red Sail plants from either production system nor from New Red Fire plants harvested from open field plots. Only $V. tricorpus$ was isolated from symptomatic plants all three years.

**Pathogen virulence.** All selected isolates of $B. cinerea$, $S. sclerotiorum$, and $V. tricorpus$ inoculated onto plants in greenhouse studies were pathogenic to lettuce. Bc.Ls.2010 caused gray mold on eight of twelve (67%) Batavia plants, Bf.Ls.2010 was pathogenic on twelve of twelve (100%) Batavia plants, and Bc.Ls.2011 infected two of five (40%) Jericho plants. Ss.Ls.2010 was pathogenic on Salinas and caused symptoms of lettuce drop in six of twelve (50%) plants, but no disease symptoms or signs were observed on cultivar Coastal Star. However on Coastal Star, Ss.Ls.2011 was pathogenic on four of eight plants (50%). The three isolates of $V. tricorpus$, Vt.Ls.2010, Vt.Ls.2011-1, and Vt.Ls.2011-2, were all virulent on lettuce but symptoms were less prominent than symptoms observed in plants inoculated with the Vd.Fs.2010 isolate (Fig. 20). Although the foliar symptoms caused by the two species were similar, vascular discoloration was
more apparent in both intensity and length in plants inoculated with Vd.Fs.2010. Sometimes vascular tissues of plants inoculated with *V. tricornutus* were either faintly or not visibly discolored but when vascular tissue was plated on agar or placed in a moist chamber, sporulation and subsequent microsclerotia formation was evident. Vt.Ls.2010 was pathogenic in trial one and infected 42% of Batavia, 17% of Coastal Star, 17% of Little Jem, 25% of Merlot, and 42% of Salinas plants. Plants inoculated with Vd.Fs.2010 also showed symptoms in all cultivars tested including 25% of Batavia, 58% of Coastal Star, 25% of Little Jem, 25% of Merlot, and 50% of Salinas plants. In trial two, Vt.Ls.2010, Vt.Ls.2011-01 and Vt.Ls.2011-02 isolates were tested for pathogenicity on Coastal Star. Vt.Ls.2010 infected 25% of plants, and both Vt.Ls.2011-01 and Vt.Ls.2011-02 infected 13% plants, while Vd.Fs.2010 infected 25% of the plants. In trial three, again all Vt.Ls.2010, Vt.Ls.2011-01 and 2011-02 isolates were tested on Coastal Star and again all were pathogenic: Vt.Ls.2010 infected 40% of plants, Vt.Ls.2011-01 infected 30%, and both Vt.Ls.2011-02 and Vd.Fs.2010 infected 20% of plants.

**Lettuce yield and quality in high tunnels vs. open fields.** No significant yield differences were observed between high tunnel and open field production systems in 2010 and 2011, but in 2012 yield was significantly (*P* = 0.0122) higher in high tunnels than in the open field. However, different cultivars were planted in 2012 than in 2010 and 2011. In 2011, marketable weight of Green Star had significantly higher marketable weight in both high tunnel (*P* < 0.0001) and open field systems (*P* = 0.0003) compared to all other cultivars.

Tip burn was not a problem in 2010 but in 2011 tip burn significantly (*P* = 0.0322) increased in high tunnel compared to open field production. Jericho, a Romaine type, had significantly (*P* = 0.0011 and *P* = 0.0001) more tip burn in high tunnels and open field plots,
respectively, compared to all other cultivars. In 2012, New Red Fire, a leaf type, had significantly ($P = 0.0114$) higher incidence of tip burn than all other cultivars in the open field.

**Discussion**

This study evaluated the use of a high tunnel production system to alter environmental conditions like leaf wetness and soil moisture as a way to control disease outbreaks in western Washington. Gray mold, lettuce drop and Verticillium wilt were observed in both high tunnel and open field experimental field production systems in 2010, 2011, and 2012. Gray mold on strawberry can be reduced in high tunnel systems (33; 92), but in this study on lettuce there was higher incidence of gray mold on lettuce inside high tunnels than in open field plots one out of three years. This discrepancy could be due to plant architecture. Lettuce leaves are in close proximity to each other and as the plant matures, lettuce heads develop whorls of leaves that trap moisture. In contrast, strawberry leaves are extended on petioles and this arrangement allows for air flow. Strawberry leaves are also waxy compared to thin lettuce leaves, and the cuticle differences may contribute to infection and latent periods of *B. cinerea* infection as reviewed by Elad and Evensen (19). Latent periods may also differ between lettuce and strawberry crops such that infection by *B. cinerea* in lettuce proceeds faster than in strawberry. Sosa-Alvarez *et al.* (70) report that latent periods in strawberry can be less than 3 days during optimal growth temperatures. However, spores of *B. cinerea* are capable of germinating and infecting vegetables within five to eight hours in the presence of high humidity (61; 98).

Since *B. cinerea* is capable of germinating and infecting hosts when relative humidity reaches 90% (5), the absence of free water alone is not sufficient to control gray mold inside high tunnels. Relative humidity inside the high tunnels was similar to that in open fields each year. A
study on lettuce in high tunnel production in Kansas also reported that relative humidity did not vary greatly between high tunnels and open fields (96). Although relative humidity may be similar in both production systems, pockets may develop more readily in high tunnels due to limited air flow. Wind speed was reduced inside tunnels as compared to the open field all three years. The severe gray mold outbreak in 2010 occurred four days after relative humidity reached 90% and when average air temperature was 14°C both inside and outside the high tunnels.

Although rainfall is eliminated in high tunnels, fog and condensation occur in the protective structures, leading to disease. In 2011, morning and afternoon fogs were common and leaf wetness within the high tunnels was higher by approximately 55 hours as compared to 2010. Although leaf wetness was higher in the open fields as compared to high tunnels in 2011, the combination of leaf wetness and higher air temperatures within the high tunnels was conducive for the growth of *B. cinerea*. Pockets of humidity which favor disease can also be created when plants are closely spaced, heads expand and rows close, high tunnels are constructed or ventilated incorrectly, or if persistent weather conditions such as fog and low temperatures prevail.

An additional factor that may contribute to infection by *B. cinerea* is tip burn, a calcium deficiency which causes necrosis along leaf margins, creating infection courts. Romaine cultivars are more likely than other cultivars to develop tip burn. In 2010, Romaine types Jericho and Coastal Star had significantly higher gray mold incidence than Boston and leaf types. Most infections by *B. cinerea* in 2011 and 2012, however, occurred in lettuce crowns and not in head tissues where tip burn is common.

In the 2010 and 2011 field studies, Green Star, a leaf type, performed exceptionally well in yield and exhibited some disease resistance to gray mold. During field ratings, gray mold
symptoms were only observed on a few Green Star plants within the high tunnels, but never on Green Star plants in the open field. At harvest, signs and symptoms of gray mold on Green Star plants were not evident for either production system. It is possible that the outer leaves exhibiting signs of sporulation had completely rotted or were inadvertently left in the field at time of harvest. In 2010 and 2011 outer leaves of mature lettuce heads were removed prior to disease assessments and therefore symptoms of gray mold on leaves with small lesions or slight rot may have been overlooked. In 2012 all outer leaves were observed for sporulation, lesions and rot. Green Star was not selected as part of that particular cultivar evaluation since other cultivars which could potentially perform well in western Washington also needed be tested. Additionally, severity ratings alone may not be an indicator of gray mold resistance because the severity ratings for Red Sails were lower on lettuce heads from open field plots while a higher incidence of diseased heads of Red Sails were harvested from high tunnel plots, 26 heads compared to seven heads from open field plots. Although there are currently no lettuce cultivars that are resistant to gray mold, there is a correlation between increased levels of lettucenin A, a phytoalexin produced by lettuce tissues, and a reduction in lesion expansion caused by \textit{B. cinerea} infection (7). Sessa \textit{et al.} (64) found that phytoalexin production can vary within lettuce cultivars; Diana produces higher amounts than the cultivar Benita. Green Star could have potentially produced higher levels of lettucenin A compared to the susceptible cultivars in the trial but production of lettucenin A was not tested.

A seedborne source of gray mold in the Romaine types cannot be ruled out. \textit{B. cinerea} has been isolated from surface-sterilized lettuce seed treated with a bleach/surfactant solution soak and water rinse (71). Additionally, isolates of \textit{B. cinerea} may be endophytic in symptomless lettuce plants and \textit{B. cinerea} is also systemic in lettuce plants (71). Although detection of \textit{B.}
cinerea on samples of seed from the Jericho and Coastal Star seed lots was attempted in 2010, B. cinerea was not recovered. However, the seed lot samples were relatively small (100 seeds).

Previous studies show that sporulation of B. cinerea is inhibited in the presence of blue-violet visible light (400 to 480 nm). When spores were cultured on PDA or tomato stems and flowers under UV-absorbing polyethylene film, sporulation was reduced compared to nonUV-absorbing film (50). Although suppressing sporulation by B. cinerea does not eliminate disease, the film could potentially delay lesion development especially since epidemics caused by B. cinerea are polycyclic (16). Future studies evaluating differences in gray mold outbreaks with various UV-absorbing plastics in high tunnel production could potentially offer additional disease management strategies for organic lettuce and vegetable production. Further, lettuce cultivar and type selection may limit gray mold in high tunnels, and become important for growers if fungicide resistance becomes problematic in B. cinerea populations.

High tunnel production systems have the potential to slow the development of lettuce drop outbreaks caused by ascospores, the primary infectious propagule of S. sclerotiorum. Sclerotia will germinate to form either mycelia or apothecia. Apothecial stipes develop and differentiate to form the apothecial disc in the presence of light as reviewed by Willetts and Wong (90) and air currents then disperse ascospores from the mature apothecia. The 0.03 mm black polyethylene mulch film used in these field studies restricts sunlight and air flow at the soil surface, except in and around planting holes. The mulch barrier could reduce apothecial formation by blocking light, or reduce ascospore dispersal. Apothecia are only fertile when developing in light that reaches or exceeds 58.2 \( \mu \text{E m}^{-2} \text{s}^{-1} \) (39). Theoretically, reducing sclerotial germination and ascospore dispersal within high tunnels could lead to healthier crops but there still remains the possibility of inoculum arriving into the high tunnel from adjacent fields.
Hammond et al. (23) report that thousands of ascospores released over multiple days may be carried up to 3-4 km. Wind speed, inside the high tunnels were reduced by 0.9 to 1.6 mph, compared to the open field.

Since symptoms of lettuce drop occurred only in lettuce crowns in 2010 and 2011, and were primarily observed in crown tissues in 2012, it is more likely that infection by S. sclerotiorum occurred via mycelial rather than ascospore inoculum in this study. Stipe tips formed during carpogenic germination require sunlight for disc differentiation, but myceliogenic germination is regulated by soil moisture and temperature (90). Lettuce drop was also observed among clusters of plants, which also implies mycelial infection. Questions regarding production system and mulch effects on sclerotia germination could be addressed in future studies which evaluate apothecia production in naturally infested soils with visual observations and spore traps.

A single application of Contans in 2011, to control S. sclerotiorum, did not appear to reduce lettuce drop. Although disease incidence was higher in 2010, the reduction in 2011 cannot necessarily be attributed to Contans because gray mold, a similar disease with similar environmental requirements, also was higher in 2010 than 2011. In addition, multiple applications are recommended for proper biocontrol but the use of polyethylene mulch precluded multiple applications. Contans cannot target ascospores introduced from external sources (23) either as the mycoparasite attacks sclerotia in the soil; however, Whipps and Gerlagh (87) review studies in which C. minitans was isolated from sclerotia of Sclerotinia spp. six to 18 months after soil applications while foliar applications of C. minitans did not lessen lettuce drop in greenhouse lettuce trials.

Reducing soilborne inoculum levels is an important part of integrated pest management programs for diseases like Verticillium wilt (4; 93), and to a lesser extent, for lettuce drop (22;
As observed in 2012, *S. sclerotiorum* is capable of producing viable sclerotia within diseased lettuce heads during the growing season. Some plants affected by lettuce drop completely rotted in the field before harvest. Thus, the sclerotia that are formed during current season infection could contribute to annual increases in inoculum densities. Selecting lettuce cultivars which are harvested prior to sclerotial development may be an important factor in disease control, especially for highly susceptible cultivars grown in high tunnels, since sclerotia were more abundant from heads harvested in this production system. All cultivars tested were susceptible to lettuce drop in all years and in 2011 and 2012, early harvested types had not yet formed sclerotia in rotting tissues. Sclerotia formation was typically observed in late-June and early-July (60 to 66 DAT) but during favorable conditions, sclerotia may be formed as early as early-June (49 DAT). For organic growers in western Washington to achieve adequate disease control of lettuce drop, the introduction of sclerotia into the soil needs to be minimized.

*Sclerotinia* has a wide host range and may survive years without a host (9; 63). Additionally, *S. sclerotiorum* is capable of undergoing sexual reproduction, which could lead to virulent strains within populations. *S. sclerotiorum* populations on pea and lentil in eastern Washington are clonal whereas isolates from lettuce in California have high recombination frequencies (45). Populations of *S. sclerotiorum* between western and eastern Washington could vary due to environment and crop differences, and comparisons between lettuce crops along coastal areas of the US could be informative.

Since lettuce was cropped in rotation with strawberry, and these crops are both susceptible to Verticillium wilt, it is likely that a build-up of soilborne inoculum contributed to the increase of symptomatic plants of Verticillium wilt between 2010 to 2011 and 2011 to 2012 in both production systems. Lettuce and strawberry crop rotations are common in California.
(78), but not in western Washington. Within three years of time in this study, symptoms of Verticillium wilt on plants harvested from high tunnel plots increased from three to 16 to 60 lettuce plants (which was 0.2, 1.2, and 4.9% of all harvested heads) to from 2010 to 2012, respectively. Symptoms of Verticillium wilt on plants harvested from open field plots also increased from nine to 111 plants (which was 0.7 and 9.1% of all harvested heads) between 2011 and 2012, respectively. Increased incidence of Verticillium wilt in 2012 may in part be a function of planting more susceptible cultivars, since different cultivars were evaluated during the 2011 and 2012 growing seasons. Two cultivars, Coastal Star and New Red Fire, were planted in all three field trials. The incidence of Verticillium wilt on Coastal Star increased each year, starting with two symptomatic heads (0.2%) in 2010, then seven symptomatic heads (0.5%) in 2011, and 92 (8%) in 2012. Symptoms of Verticillium wilt on New Red Fire were only observed in 2012 on eight plants (0.8%), all harvested from high tunnel subplots.

\textit{V. dahliae} was present within the field study site based on isolations from strawberry and soil assays that recovered from 0 to 13 cfu per gram (Appendix B), but \textit{V. dahliae} was never recovered from symptomatic field-grown lettuce. Instead, \textit{V. tricorpus} was always recovered. Greenhouse studies showed that the Vd.Fs.2010 isolate is pathogenic on lettuce, and more virulent than the \textit{V. tricorpus} isolates obtained from the lettuce tissues. It is possible that \textit{V. tricorpus} is able to colonize lettuce more readily than \textit{V. dahliae}, thereby interfering with \textit{V. dahliae} infection. In other studies on lettuce, co-inoculation with both \textit{V. tricorpus} and \textit{V. dahliae} resulted in disease, but severity was reduced (58). In those cases, plants were inoculated in the greenhouse but conditions for infection in the field in this study may have favored one pathogen over the other. There may also be inoculum threshold differences for infection by \textit{V. tricorpus} compared to \textit{V. dahliae}. Atallah et al. (4) estimated (based on a previous study by (82)
that over one million microsclerotia are produced from a single plant infected by *V. dahliae*. As cited by Attallah *et al.* (4), *V. dahliae* infection on lettuce requires an unusually high inoculum density (over 100 microsclerotia per g of soil) compared to other susceptible crops such as strawberry (about 3 microsclerotia per g of soil) (25), cauliflower (four to 10 microsclerotia per g of soil) (91), or potato and mint (about 10 microsclerotia per g of soil) (51). Since *Verticillium* wilt on lettuce caused by *V. tricorpus* does not cause significant yield loss, threshold studies have not yet been done. Due to the primarily saprophytic lifestyle of *V. tricorpus*, it is likely that this fungus could be continuously active as compared to *V. dahliae*, which may be dormant or just beginning to germinate at times when infection has already been favorable for *V. tricorpus*. Although *V. dahliae* may have periods of dormancy, plant exudates from any species can stimulate germination (8). Furthermore, high tunnel cropping systems, which increase soil temperature, may affect infection by *Verticillium* spp. in lettuce crops. *V. tricorpus* has an optimal growth temperature at 24°C on Czapek-Dox agar and colonies grow between 15 to 30°C (42), whereas isolates cultured on PDA grew optimally at 20°C and were inhibited at 30°C (58). Maximum growth of *V. dahliae* cultured on PDA occurs at 25°C (58) and some *V. dahliae* isolates can grow at 30°C (55). Average soil temperatures for open field and high tunnel plots in 2010, 2011, and 2012 were 15.0 and 17.4°C, 17.0 and 17.7°C, and 15.6 and 16.3°C, respectively. The cooler soil temperatures may have favored *V. tricorpus* growth and infection over *V. dahliae*.

Vegetative compatibility groups (VCG) of *Verticillium* spp. can sometimes be linked to host specificity and virulence. Therefore, infection of lettuce by *Verticillium* populations in western Washington field soils may have also been affected by differences in the VCG present. Although three isolates of *V. tricorpus* and one isolate of *V. dahliae* were tested for pathogenicity...
on lettuce in repeated greenhouse trials, VCG testing was not completed on any of the isolates obtained during the field studies. Studies on VCG infecting lettuce are not complete (18) and future studies should evaluate potential pathogenicity differences among the nine VCG groups identified (42). VCG testing among *V. tricorpus* isolates should include mildly pathogenic isolates on lettuce as reported in California (58) and Washington as well as virulent isolates from lettuce reported from Japan (77).

High tunnels reduce leaf wetness, a contributing factor to many foliar diseases (30; 89). Lettuce production has benefited from high tunnel structures for crop establishment in Canada and in many other areas of the United States due to early production and increased quality (13; 59; 81; 89). High tunnels provide an attractive alternative to chemical disease control for both organic and conventional farmers. Disease incidence and severity of gray mold, lettuce drop, and *Verticillium* wilt did not translate into loss of yield for either production system in 2010, 2011, or 2012. However, lettuce production under high tunnels can have disadvantages as well. In this study high tunnels reduced leaf wetness near head maturation and increased temperatures by approximately 1°C in all three years, but the structures did not allow for sufficient ventilation to prevent pockets of high relative humidity. Future studies should look at disease incidence when high tunnels are ventilated during high temperature and humidity periods compared to venting only when temperatures exceed a specific threshold.

High tunnels add to production costs that can vary depending on materials used. In 2003 Waterer (85) reported high tunnel material costs to be $13.25/m of row which is similar to reports by Wells and Loy (86). Washington growers may choose to offset high tunnel material costs with continuous cropping of high value vegetable crops such as fresh market lettuce. However, cycles of continuous cropping could lead to build-up of soilborne inoculum and has
the potential to cause severe crop loss for future seasons. In certain areas of western Washington, lettuce rotations are not practiced because urbanization has reduced available acreage (31). The Natural Resources Conservation Service offers financial assistance of up to $300,000 through its Environmental Quality Incentives Program (EQIP) to growers who utilize seasonal high tunnels in many states of the U.S. (80). EQIP is tracking pesticide use, energy expenditure, and soil quality within high tunnel production systems and one EQIP area of focus is on conservation crop rotation. Qualified growers in western Washington might apply for this type of financial assistance and, under EQIP rules, start crop rotations or organically approved management practices with lettuce.

The cultivar evaluations completed in this study show that Green Star is an ideal loose leaf lettuce for western Washington. Green Star had relatively high yields, showed some resistance to gray mold, and was not seriously affected by either lettuce drop or Verticillium wilt. The results from the cultivar evaluations in 2012 also indicate that Coastal Star is prone to all three fungal diseases, but Nevada has some potential for limiting outbreaks of gray mold, lettuce drop and Verticillium wilt. Other cultivars should continue to be evaluated for the purpose of commercial production in western Washington, especially those with an upright growth habit such that outer leaves do not drop and create a seal between the plant and the mulch at the planting hole.
Literature Cited:


TABLE 7. Lettuce type and cultivar planted during field trials evaluating lettuce cultivars grown in high tunnel and open field plots near Mount Vernon, WA.

<table>
<thead>
<tr>
<th>Year</th>
<th>Lettuce type</th>
<th>Cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010 and 2011</td>
<td>Boston</td>
<td>Adriana&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ermosa&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>Green Star&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>New Red Fire&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Romaine</td>
<td>Coastal Star&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jericho&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>2012</td>
<td>Crisphead</td>
<td>Concept&lt;sup&gt;z&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nevada&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>New Red Fire&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Red Sails&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Romaine</td>
<td>Coastal Star&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valmaine&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>x</sup> Seeds supplied from Territorial Seed (Cottage Grove, OR).
<sup>y</sup> Seeds supplied from Jonny's Selected Seed (Windslow, ME).
<sup>z</sup> Seeds supplied from Seeds of Change (Rancho Dominguez, CA).
TABLE 8. Dates for planting lettuce, constructing high tunnels, and fertigating during experimental field trials evaluating lettuce cultivars grown in high tunnels and open fields near Mount Vernon, WA.

<table>
<thead>
<tr>
<th>Year</th>
<th>Date seeded&lt;sup&gt;v&lt;/sup&gt;</th>
<th>Date transplanted</th>
<th>Date high tunnel constructed&lt;sup&gt;w&lt;/sup&gt;</th>
<th>Fertilizer application&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Irrigation schedule&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Fertigation schedule&lt;sup&gt;z&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>11 Mar</td>
<td>22 Apr</td>
<td>19 Apr</td>
<td>7 Apr</td>
<td>22 Apr-1 Jul</td>
<td>7 May-4 Jun</td>
</tr>
<tr>
<td>2011</td>
<td>10 Mar</td>
<td>22 Apr</td>
<td>29 Apr</td>
<td>20 Apr</td>
<td>22 Apr-28 Jun</td>
<td>16 May-14 Jun</td>
</tr>
<tr>
<td>2012</td>
<td>12 Mar</td>
<td>26 Apr</td>
<td>20 Apr</td>
<td>17 Apr</td>
<td>26 Apr-9 Jul</td>
<td>21 May-19 Jun</td>
</tr>
</tbody>
</table>

<sup>v</sup> Two lettuce cultivars, Nevada and Valmaine, were seeded on 5 Apr.

<sup>w</sup> Open-ended high tunnels (’Solo’ model, Haygrove LTD, UK; 36.6 m long x 8.4 m wide x 3.0 m high) were replicated four times.

<sup>x</sup> Fertilizers Par4 9-3-7 organic (North Pacific Ag Products, Portland, OR) in 2010 and 2011 and Proganic 8-2-4 (Wilbur Ellis, Wenatchee, WA) in 2012 were broadcast applied at 90 kg N/ha.

<sup>y</sup> In 2010 and 2011 irrigation was applied at 63.5 mm twice per week, but in 2012 irrigation was spread out at 25.4 mm five times per week.

<sup>z</sup> Three fertigation events with Converted Organics 521 (Converted Organics of California LLC, Gonzales, CA) totaled 133, 54, and 27 g of N, P, and K respectively in 2010 and 2011 but was reduced to 95, 38, and 19 g of N, P, and K, respectively, in 2012.
TABLE 9. Comparison of disease onset and time to harvest for lettuce cultivars in high tunnel and open field production systems near Mount Vernon, WA based on days after transplanting. Experimental field trials were conducted during the spring, from 2010 through 2012.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Gray mold disease symptom onset</th>
<th>Lettuce drop disease symptom onset</th>
<th>Harvest date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High tunnel</td>
<td>Open field</td>
<td>High tunnel</td>
</tr>
<tr>
<td>Adriana</td>
<td>49</td>
<td>48 / 49</td>
<td>- / -</td>
</tr>
<tr>
<td>Coastal Star</td>
<td>49</td>
<td>48 42 / 49</td>
<td>57 55 / 56</td>
</tr>
<tr>
<td>Concept</td>
<td>/ 28 / -</td>
<td>50 / -</td>
<td>- / 48</td>
</tr>
<tr>
<td>Greenstar</td>
<td>49 55 / -</td>
<td>55 / 57</td>
<td>48 / -</td>
</tr>
<tr>
<td>Nevada</td>
<td>/ 42 / -</td>
<td>50 / -</td>
<td>- / -</td>
</tr>
<tr>
<td>Red Fire</td>
<td>49 48 28 / 49 48 54</td>
<td>57 - 54 / 57 48 54</td>
<td>54-57 49-55 54-57 57-60 49-60 54-57</td>
</tr>
<tr>
<td>Red Sails</td>
<td>/ 42 / -</td>
<td>42 / -</td>
<td>- / -</td>
</tr>
<tr>
<td>Valmaine</td>
<td>/ 50 / -</td>
<td>56 / -</td>
<td>- / -</td>
</tr>
</tbody>
</table>

/ = indicates that the cultivar was not tested during the crop year.
- = disease was not observed during weekly field ratings.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>High tunnel</td>
<td>11.5</td>
<td>6.1 a</td>
<td>---</td>
<td>3.9</td>
<td>3.2</td>
<td>9.8 a</td>
<td>2.6</td>
<td>9.4</td>
</tr>
<tr>
<td>Open field</td>
<td>3.1</td>
<td>1.8 b</td>
<td>---</td>
<td>5.6</td>
<td>2.7</td>
<td>2.2 b</td>
<td>1.4</td>
<td>18.3</td>
</tr>
<tr>
<td>P Values</td>
<td>0.0639</td>
<td>0.0037</td>
<td>---</td>
<td>0.759</td>
<td>0.857</td>
<td>&lt;.0001</td>
<td>0.102</td>
<td>0.5123</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>High tunnel</th>
<th>Open field</th>
<th>High tunnel</th>
<th>Open field</th>
<th>High tunnel</th>
<th>Open field</th>
<th>High tunnel</th>
<th>Open field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adriana</td>
<td>0.5 b a</td>
<td>7.6 a</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>5</td>
<td>3.2</td>
</tr>
<tr>
<td>Coastal Star</td>
<td>12.4 a</td>
<td>4.8 a</td>
<td>55.4 a</td>
<td>4.5 b</td>
<td>10.9</td>
<td>3.4</td>
<td>10.8</td>
<td>3.5 ab</td>
</tr>
<tr>
<td>Concept</td>
<td>/</td>
<td>/</td>
<td>28.2 b</td>
<td>3.6 b</td>
<td>/</td>
<td>/</td>
<td>5.5</td>
<td>/</td>
</tr>
<tr>
<td>Ermosa</td>
<td>1.4 b</td>
<td>6.9 a</td>
<td>/</td>
<td>/</td>
<td>2.9</td>
<td>4.1</td>
<td>/</td>
<td>1.0 ab</td>
</tr>
<tr>
<td>Green Star</td>
<td>0.0 b</td>
<td>0.0 b</td>
<td>/</td>
<td>/</td>
<td>0.9</td>
<td>1.8</td>
<td>/</td>
<td>1.4 cd</td>
</tr>
<tr>
<td>Jericho</td>
<td>29.5 a</td>
<td>1.8 ab</td>
<td>/</td>
<td>/</td>
<td>1.8</td>
<td>1.8</td>
<td>/</td>
<td>3.6 a</td>
</tr>
<tr>
<td>Nevada</td>
<td>/</td>
<td>/</td>
<td>20.5 b</td>
<td>0.0 b</td>
<td>/</td>
<td>/</td>
<td>3.2</td>
<td>/</td>
</tr>
<tr>
<td>New Red Fire</td>
<td>0.0 b</td>
<td>2.9 a</td>
<td>19.1 b</td>
<td>3.6 b</td>
<td>6.9</td>
<td>3.2</td>
<td>10.8</td>
<td>0.0 d</td>
</tr>
<tr>
<td>Red Sails</td>
<td>/</td>
<td>/</td>
<td>23.4 b</td>
<td>24.1 a</td>
<td>/</td>
<td>/</td>
<td>1.3</td>
<td>/</td>
</tr>
<tr>
<td>Valmaine</td>
<td>/</td>
<td>/</td>
<td>25.2 b</td>
<td>0.0 b</td>
<td>/</td>
<td>/</td>
<td>4.4</td>
<td>/</td>
</tr>
<tr>
<td>P Values</td>
<td>&lt;.0001</td>
<td>0.0072</td>
<td>0.0004</td>
<td>&lt;.0001</td>
<td>0.8157</td>
<td>0.679</td>
<td>0.0625</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

*Verticillium wilt percent incidence data not analyzed in 2010 because symptoms were not recorded until final harvest, after several cultivars had already been harvested.

No significant interaction between location and cultivar were detected in 2010 and 2011 but in 2012 a significant interaction was detected for gray mold incidence only.

Numbers within a column followed by the same letter are not significantly different (P=0.05) as determined by Fisher's protected LSD.

Percent incidence values back-transformed from rank transformation for presentation.

Percent incidence values back-transformed from reciprocal transformation for presentation.

/ = indicates that the cultivar was not tested during the crop year.
### TABLE 11. Recovery, viability, and size of sclerotia produced in lettuce tissue infected by *Sclerotinia sclerotiorum* from harvested lettuce plants grown in experimental plots near Mount Vernon, WA in 2012.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Number affected</th>
<th>Sclerotia recovered</th>
<th>Sclerotia viability</th>
<th>Sclerotia size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Weight (g)</td>
<td>% viable†</td>
</tr>
<tr>
<td>Coastal Star</td>
<td>7</td>
<td>606</td>
<td>15.17</td>
<td>56†</td>
</tr>
<tr>
<td>Concept</td>
<td>2</td>
<td>67</td>
<td>1.38</td>
<td>50</td>
</tr>
<tr>
<td>Nevada</td>
<td>2</td>
<td>106</td>
<td>1.47</td>
<td>0</td>
</tr>
<tr>
<td>New Red Fire</td>
<td>2</td>
<td>67</td>
<td>1.27</td>
<td>0</td>
</tr>
<tr>
<td>Red Sails‡</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Valmaine</td>
<td>5</td>
<td>548</td>
<td>7.45</td>
<td>60</td>
</tr>
</tbody>
</table>

† Total number of harvested heads with sclerotial formation by *S. sclerotiorum*.  
‡ Percent of viable sclerotia obtained randomly by selecting five sclerotia from each plant and plating each sclerotium onto ½ PDA with bromophenol blue, and confirming color change and sclerotial production.  
§ Total number of sclerotia produced in culture from viable sclerotia plated onto ½ PDA with bromophenol blue.  
‖ Small = sclerotia from each plant, minus five selected for viability, sized by US No. 25 sieve (up to 710 µm).  
‖ Intermediate = sclerotia from each plant, minus five selected for viability, sized by US No. 12 sieve (711 µm to 1.70 mm).  
‖ Large = sclerotia from each plant, minus five selected for viability, sized by US No. 6 sieve (1.71 mm to 3.35 mm).  
‡ Lettuce drop observed on one Red Sails plant during weekly field ratings; three sclerotia were observed in crown tissues and were plated onto ½ PDA with bromophenol blue, but plant was completely rotted by the time the subplot was harvested.
Fig. 10. Gray mold symptom severity scale on lettuce plants harvested from high tunnel and open field plots near Mount Vernon, WA in 2010, 2011 and 2012. A, Lesion on one leaf = 1% severity; B, some rot on three outer leaves and slight sporulation = 5% severity; C, one side of lettuce head affected, some rot and sporulation visible = 20% severity; D, most of crown infected, rot, wilt, chlorosis, and sporulation are visible = 50% severity; and E, crown and head infected, rot and sporulation in crown and wilt, chlorosis and necrosis in head tissue = 70% severity; F, whole plant dead, rotted tissues have become dry = 100% severity.
**Fig. 11.** Lettuce drop symptom severity scale on heads harvested from high tunnel and open field production systems near Mount Vernon, WA in 2010, 2011, and 2012. Severity of infection by *S. sclerotiorum* assigned **A**, 20% because infection progressed into inner tissues; **B**, 40% since most of the crown is infected, white mycelia are visible in crown tissues and rot has moved into portions of the head; **C**, 60% with the whole crown rotted and mycelium visible in head tissues with most of the plant wilted and rot starting to develop in inner leaves; **D**, 70% due to multiple areas rotted and visible sclerotia in crown tissues; **E**, 100% if whole plants had collapsed, leaves congealed, and crown rotted completely, or **F**, whole plant had dried up and black sclerotia were visible throughout tissues.
Fig. 12. Symptoms of Verticillium wilt on harvested lettuce grown near Mount Vernon, WA in high tunnel and open field productions systems. A, patterns of either v-notch or one-sided chlorosis on a few outer leaves; B, one-sided chlorosis and stunted growth (plant on right) compared to healthy plant (on left); C, one-sided vascular discoloration in crown tissues, as seen at harvest; and D, crown tissue with discolored vascular tissue placed in a moist chamber to isolate Verticillium sp.
Fig. 13. Tip burn observed on various lettuce cultivars grown near Mount Vernon, WA in high tunnel and open field plots during the 2010 through 2012 growing seasons. A, Tip burn on outer leaf from a marketable head; B, slight tip burn in lettuce head tissue, rendering the head unmarketable; C, severe tip burn extending down the leaf margin in outer leaves reducing head size; D, severe tip burn in Romaine cultivar Coastal Star.
Fig. 14. Daily average air temperatures from 96 measurements per day in 15-minute intervals recorded by Hobo U-30 weather monitoring system in high tunnel (HT) versus open field plots (OF) at experimental field trial near Mount Vernon, WA in A, year 2010; B, year 2011; and C, year 2012.
Fig. 15: Comparisons between high tunnel (HT) and open field (OF) production systems for rainfall and leaf wetness during three consecutive lettuce growing seasons (April to June, 2010 to 2012). **A**, precipitation and **B**, leaf wetness is accumulated hours that leaf surfaces reached or exceeded a wetness threshold of conductivity above 50.
Fig. 16: Photosynthetically active radiation (PAR) comparisons between high tunnel (HT) and open field (OF) plots at experimental trial near Mount Vernon, WA. Daily PAR is given as an average of 96 readings per day in 15-minute increments in A, year 2010; B, year 2011; and C, year 2012.
Fig. 17: Percent relative humidity inside (HT) and outside (OF) high tunnels during spring lettuce growing seasons near Mount Vernon, WA as averaged across 96 recordings per day in 15-minute intervals in A, year 2010; B, year 2011; and C, year 2012.
Fig. 18. Daily soil temperatures recorded at 5 cm depth within 23-cm raised beds covered with polyethylene mulch in an experimental trial near Mount Vernon, WA comparing high tunnel (HT) and open field (OF) plots with spring lettuce. Averages obtained from 96 recordings per day in 15 minute intervals in A, year 2010; B, year 2011; and C, year 2012.
Fig. 19. Average daily wind speeds recorded in 15-minute intervals by a Hobo weather monitoring system located in a row within a high tunnel (HT) and open field (OF) plot near Mount Vernon, WA. Comparisons made between years in A, 2010; B, 2011; and C, 2012.
Fig. 20. Vascular discoloration of greenhouse-inoculated lettuce plants. A, cultivar Merlot inoculated with strawberry isolate of *V. dahliae* to compare symptoms with B, Merlot inoculated with lettuce isolate of *V. tricorp*us and C, susceptible cultivar Salinas inoculated with the same *V. dahliae* and D, the same *V. tricorp*us isolates from 2010.
APPENDIX A

PROCEDURES FOR ISOLATING NATIVE SOIL MICROORGANISMS
FROM BIODEGRADABLE PLASTIC FILMS

This appendix covers the laboratory techniques and protocols used to isolate and assess the potential of select native soil microflora to degrade the agricultural mulches researched in a USDA-AFRI SCRI-SREP project (Grant Award No. 2009-51181-0587) in 2010 as part of a ‘mesh bag’ study. These protocols were designed by Dr. Brodhagen. I put them into text form as Standard Operating Procedures (SOPs), for my use and that of other project personnel, during my internship, Spring 2011, in Dr. Brodhagen’s microbiology laboratory at the USDA-ARS Forage Seed and Cereal Research Unit in Corvallis, OR. The procedures were posted on the project team’s internal website at the conclusion of my time in Corvallis. The procedures were amended later to address additional testing of oligotrophs, and revised and submitted in September 2012 as a refereed journal article (See: Bailes, G., Lind, M., Ely, A., Powell, M., Moore-Kucera, J., Miles, C., Inglis, D., and Brodhagen, M. 201x. Isolation of native soil microorganisms with potential for breaking down biodegradable plastic films used in agriculture. Journal of Visualized Experiments (accepted; in press; to be published online). The SOPs are separated into three sections which include (i) mulch suspension dilution plating, (ii) media preparation, and (iii) microbe biodegradation capability assays.
I. MESH BAG MULCH EXTRACTION: MULCH SUSPENSION

DILUTION PLATING AND SOIL/MULCH STORAGE

**General approach.** The biodegradable mulches (BDMs) must be extracted from the soil within the mesh bags and sampled such that small pieces can be agitated in liquid to suspend microbes adhering or embedded in mulch. Serial dilutions are made to separate the various microbes present in solution to single cells or colony forming units (CFUs). Culture the organisms surrounding the BDMs by spreading dilution solutions onto selective media and incubating at 20°C for five days. Also keep a portion of the mulch and soil samples, processing them for long-term storage and potential further research.

**Materials required**

<table>
<thead>
<tr>
<th>Process in dirt lab</th>
<th>Process in clean lab/sterile hood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Box of latex gloves</td>
<td>400 mL Phosphate buffered saline (PBS)</td>
</tr>
<tr>
<td>25 Large weigh boats</td>
<td>40  25 mL culture tubes with caps (autoclaved)</td>
</tr>
<tr>
<td>5 Small weigh boats</td>
<td>80 1/10 X TSY + cyclohexamide (50 ug/ml) plates</td>
</tr>
<tr>
<td>2 Spatulas</td>
<td>80 PDA + chloramphenicol (30 ug/mL) plates</td>
</tr>
<tr>
<td>1 Pair of scissors</td>
<td>50 Small culture tubes (autoclaved)</td>
</tr>
<tr>
<td>5 Razor blades</td>
<td>1 Box of P1000 pipette tips</td>
</tr>
<tr>
<td>1 Pair of forceps</td>
<td>2 Boxes of P200 pipette tips</td>
</tr>
<tr>
<td>1 Spray bottle with 70 % EtOH</td>
<td>1 P1000 pipette</td>
</tr>
<tr>
<td>1 100 mL bottle with 95 % EtOH</td>
<td>1 P200 pipette</td>
</tr>
<tr>
<td>1 Set of paper towels</td>
<td>1 P200 multipipetter</td>
</tr>
<tr>
<td>1 Box of Kim wipes</td>
<td>2 96-well plates</td>
</tr>
<tr>
<td>20 Small paper bags (pre-labeled-dry soil)</td>
<td>1 Vortexer</td>
</tr>
<tr>
<td>20 Conical tubes (pre-labeled-wet soil)</td>
<td>1 Sonicator</td>
</tr>
<tr>
<td>16 15 mL centrifuge tubes (pre-labeled)</td>
<td></td>
</tr>
<tr>
<td>2 Balances (top loading for soil and analytical for mulch)</td>
<td></td>
</tr>
</tbody>
</table>

**Labels.** Labels for each sample should include the location where sample came from, the production system (HT; high tunnel or OF; open field), mulch treatment (BA, BioAgri; BT, BioTelo; SB, spunbond poly(lactic) acid (PLA); CC, cellulose mulch; or WG, WeedGuardPlus), which replication (A = 1, B = 2, C = 3, and D = 4), also if sample contains soil or mulch, the weight of item, and date processed. To save time, label treatment by color: pink = BA, orange =
BT, green = CC, and a black line = SB. Example: TN/HT/BA; A; Soil 20 g; air dried 3/18/2011.

**Mulch suspension dilution.** A total of 20 mesh bags (four replications—labeled A-D of each of the five treatments, including all mulches plus a no mulch control, each bag filled with approximately 300 g of soil arrive from TX, TN and WA on blue ice, and are immediately stored at 4°C overnight. Mesh bags are removed from shipping box and arranged by treatment on the bench. The treatments are processed in the following order: no mulch, cellulose, spunbond PLA, BioAgri and BioTelo. The mesh bags are removed from individual plastic bags and placed on a large plastic weigh boat. It is important that clean weigh boats are used for each treatment and that spatulas, forceps, scissors, gloves, and bench spaces are cleaned with 70% ethanol between treatments to avoid cross contamination during handling.

Mesh bags are cut with a razor blade along three sides (Fig. A1.1A) and the mesh is peeled back to expose the soil. Sterilized spatulas (dipped in 95% EtOH) are used to gently dig into the soil until the mulch piece(s) are recovered. Mulch piece(s) are lifted from the soil with sterilized forceps (Fig. A1.1B), and cut into small squares (approximately 1 x 1 cm) with sterilized scissors. Mulch pieces are weighed out to 0.5 g on small weigh boats (Fig. A1.1C) and then transferred to 15 mL culture tubes containing 9.5 mL of PBS broth (Fig. A1.1D). If the mulch has been completely degraded or you are processing the no mulch treatment, add 0.5 g of soil from the center of the mesh bag to the broth. For samples that are completely degraded, choose soil that is still discolored from the residual mulch.

The culture tubes containing 9.5 mL phosphate-buffered saline broth (PBS) and 0.5 g mulch are vortexed for 30 sec at high speed, sonicated for 10 min (Fig. A1.1E and F), and vortexed again for 30 sec. The meniscus of the solution should be just below the cap while
vortexing. This effect is achieved by holding the tube tightly at the desired meniscus height, keeping one finger slightly pressed on top to prevent solution from spilling over (Fig. A1.1F). The agitation should achieve two goals: 1) suspend microbes in solution by breaking up biofilms and physically removing cells imbedded or adhering to mulch pieces, and 2) create a homogeneous solution so that cultures are an accurate reflection of the microbes that are present (i.e., culturable on selected media; Fig. A1.1G). The microbial solution is concentrated, and must be diluted in order to obtain individual cells or CFUs (pure cultures); therefore, serial dilutions are done in a laminar flow hood (Fig. A1.2). Tubes 1 and 2 may be prepared the night before (filled with 9.5 and 4.5 mL PBS, respectively) as well as being autoclaved and pre-labeled. During sonication, prepare well plates by adding 450 µl of PBS to five wells for each replicate sample (Fig. A1.2). It is important to mix each tube/well before completing each dilution transfer. If microbes sediment in the solution, the CFUs counts and isolates will not accurately reflect what is in the mulch suspension.

One-half of a gram of mulch is weighed into Tube 1 (9.5 mL of PBS solution), making a 20-fold dilution \(5.0 \times 10^{-2}\). After sonication and vortexing (described above), 0.5 mL of solution is transferred to the 4.5 mL PBS in culture Tube 2 (Fig. A1.3A), making a \(5.0 \times 10^{-3}\) dilution. The remaining 10-fold dilutions are all made by adding 50 uL of the previous dilution to the next microplate well, which contains 450 uL PBS. Thus, Well 5 in the microplate will be a \(5 \times 10^{-8}\) dilution. In all, two dilution tubes and four microplate wells are used for each sample. Mixing between dilutions is done by vortexing culture tubes for 30 sec or by pipetting the liquid within the microplate wells up and down ten times (vortexing, unless very gentle, is liable to spread droplets from one sample well to another, and contaminate them).
For efficient diluting, first make all culture tube dilutions \((5.0 \times 10^{-2} \text{ and } 5.0 \times 10^{-3})\), and then transfer 50 uL from Tube 2 into Well 1 for sample replicates A-D. Use a multipipetter to carry out remaining transfers (Fig. A1.3B). Use one set of pipette tips to transfer 50 uL of mixed solution from Well 1 to Well 2, unload the multipipetter, and use new tips to mix solution in Well 2 and so on (Fig. A1.4). Well solutions are mixed by pipetting the solution up and down: i.e., place tips along the side of the well, release solution so that it flows down the well wall and back into solution, and repeat ten times.

After dilutions are made (Tube 1 to Well 5), 100 uL of the mulch suspension is transferred onto selective media (Fig. A1.5A). Fungal isolates are obtained by plating 100 uL from Tube 2 and Wells 1-3 onto potato dextrose agar plates containing chloramphenicol (30 ug/mL). Bacterial cultures are obtained from Wells 2-5 by plating 100 uL onto 1/10 X trypticase soy yeast (TSY) agar containing cycloheximide (50 ug/mL). The agar plates are pre-labeled, and placed in the hood one day before dilutions are made. Air dries out the agar, allowing the mulch suspension to seep in. The diluted mulch suspension should be quickly spread so that growth is uniform across the plate. Small culture tubes can be autoclaved and used to quickly spread the solution (Fig. A1.5B). The same spreader and pipette tips can be used to transfer and spread dilutions if you start with the most diluted well and work towards the less diluted well/tube.

The plates are stored upright for 30 minutes to let liquid soak in, then inverted, and incubated at 20°C in dark for five days. Eventually, dilution plates will be counted (Fig. A1.5C) and isolates from the dilution plates will be streaked onto BDMs to test for potential biodegradation capability (see first BDM test protocol).
Soil and mulch long term storage. Soil and mulch pieces for long term storage are also processed in the dirt lab during the procedure to extract mulch samples from mesh bag replicates. An additional 0.5 g of mulch is transferred to a 15 mL conical tube for permanent storage (-20°C). There are four replicates so each treatment will have four tubes containing mulch labeled A-D. Two soil samples are also prepared for permanent storage: approximately 20 g of wet soil is weighed for each sample. The first soil sample is stored wet in a conical tube, the second soil sample is transferred to a paper bag, air dried in a chemical fume hood for seven days, sealed with tape, and stored in ziplock bags. Mulch and soil samples are stored at -20°C, soil samples are kept in labeled zip-locks and double bagged.

II. MEDIA PREPARATION FOR MESH BAG STUDY

Nonselective media for microbe isolation from buried mulch pieces

For bacteria

\[ \text{I/10 X TSY (pH 7)} \]
890 mL deionized H₂O
3 g tryptic soy
0.1 g yeast extract
Adjust pH to 7.0, then bring volume up to 1 L, add
16 g agar
Autoclave, cool to \(< 50°C\), add
1.0 mL cycloheximide (50 mg/mL stock solution)

For fungi

\[ \text{PDA (pH 5)} \]
40.0 g potato dextrose agar + 1 L deionized water
Autoclave and adjust to pH 5

Minimal media for biodegradation assay

For bacteria

\[ \text{M9 0 Carbon} \]
790 mL deionized H₂O
16 g Bacto agar
Autoclave, cool to \(< 50°C\) then add:
200 mL 5X Minimal Media Salts (1 liter solution; autoclave salts 20 minutes at 15 PSI)
34 g \( \text{Na}_2\text{HPO}_4 \)
15 g \( \text{KH}_2\text{PO}_4 \)
5 g \( \text{NH}_4\text{Cl} \)
2.5 g \( \text{NaCl} \)
15 mg \( \text{CaCl}_2 \)
0.1 mL 0.5% Thiamine (Vitamin B1)
1.0 mL 1M \( \text{MgSO}_4 \)
1.0 mL cycloheximide (50 mg/ml stock solution)

\textit{M9 top agar}: as above but reduce agar to 8 g per liter
\textit{M9 liquid media}: as above but no agar
\textit{M9 plus glucose}: as above but add 5 mL glucose stock solution (0.2 %; w/v) per liter after autoclaving

\textbf{For fungi}
\textit{FMM 0 Carbon}
850 mL deionized H\(_2\)O
50 mL 20X Nitrate salts
120 g \( \text{NaNO}_3 \)
10.4 g KCl
10.4 g \( \text{MgSO}_4*7\text{H}_2\text{O} \)
30.4 g \( \text{KH}_2\text{PO}_4 \)
1 mL Trace elements
2.2 g \( \text{ZnSO}_4*7\text{H}_2\text{O} \)
1.1 g \( \text{H}_3\text{BO}_3 \)
0.5 g \( \text{MnCl}_2*4\text{H}_2\text{O} \)
0.5 g \( \text{FeSO}_4*7\text{H}_2\text{O} \)
0.16 g CoCl\(_2*5\text{H}_2\text{O} \)
0.16 g CuSO\(_4*5\text{H}_2\text{O} \)
0.11 g \((\text{NH}_4)_6\text{Mo}_7\text{O}_{24}*4\text{H}_2\text{O} \)
5.0 g Na\(_4\text{EDTA} \) (or 4.4 g Na\(_2\text{EDTA} \))
Adjust to pH to 6.5 (HCl and NaOH), then adjust solution to 1 L
16 g agar
Autoclave, cool to \( \leq 50^\circ\text{C} \) then add
1.0 mL chloramphenicol (30 mg/mL)

\textit{FMM top agar}: as above but reduce agar to 8 g
\textit{FMM liquid media}: as above but no agar
\textit{FMM plus glucose}: as above but add 10 g dextrose before autoclaving

\textbf{BDM sterilization and BDM minimal media preparation.} One BDM mulch sample is cut into small squares (4.25 x 4.25 cm) with a paper cutter and the pieces are stored in a glass beaker. BDMs are sterilized in a biohazard hood with UV-C light. The hood is sprayed down
with 70 % EtOH and then UV-sterilized for two hours. The mulch pieces are then placed in the hood, in rows. The BDM squares undergo UV light sterilization for two hours per side.

The pieces are then turned over with sterilized forceps (autoclaved tools work best because flames cannot be close to the BDMs). Work from front to back (flip near you first) so that when your arm extends toward the back, the piece that is facing up is not sterilized yet. Turn on UV light for an additional hour. Remove the mulch pieces from the hood with sterilized forceps, and place them into autoclaved beakers (cooled to room temperature).

Transfer UV-sterilized mulch pieces in the autoclaved beakers to the laminar flow hood. Place beaker with mulch pieces near the air and lay the beaker on one side. Use sterilized forceps to grab one piece of mulch and with the other hand quickly open the agar plate and place the mulch piece on top of the agar. Some mulches lay easily (cellulose, spunbond PLA) but others may require a set of forceps to lay flat (Fig. A1.6). After the mulch has been placed on agar, re-seal unused pieces in autoclaved beaker and store in cupboard under ambient conditions.

Top agar is then microwaved at 50% power in 30 second increments to avoid boiling over. The top agar is prepared in advance and stored in a cupboard (described above). Use a P200 pipette to transfer 10 uL of top agar in a 3 x 3 grid across the mulch. One drop from the P200 tip is approximately 10 uL. You do not need to touch the tip of the pipette to the mulch. Be careful to not transfer large dots because excess weight on the mulch can pull the mulch off the agar surface when the plates are stored/incubated as plates are inverted during this process.

III. MICROBE BIODEGRADATION CAPABILITY ASSAY:

FIRST AND SECOND BDM TRIALS
**General approach.** Dilution plates obtained from the mesh bag study are counted to obtain CFUs numbers and isolates are streaked onto media containing minimal nutrients with biodegradable mulch pieces as the sole source of carbon (C). BDM degraders are identified and differentiated from autotrophs by comparing growth on minimal media containing no carbon, glucose and BDMs in the first BDM trial (see below). Microbes capable of growing on the BDM surface are considered potential biodegraders of the mulch. Putative mulch degraders are streaked to single isolated colonies, then re-inoculated onto minimal media plus BDM to confirm growth in the second BDM trial. Isolates are cultured for maximum growth so that cells/spores can be stored in permanent cultures at both 80°C and 4°C.

**Materials required**

Per sample (i.e. TN/HT)

- 600 Flat tipped toothpicks (autoclaved)
- 40 Cotton swabs (autoclaved)
- 30 Long wooden applicator sticks (autoclaved)
- 20 UV sterilized mulch squares (5 x 5 cm) of each: BioAgri, BioTelo, and Spunbond PLA in sterilized containers
- 20 Autoclaved mulch pieces of cellulose control (5 x 5 cm)
  - 1 L Liquid N₂
  - 2 Pairs of forceps
  - 1 Box of P200 pipette tips
  - 1 P200 pipette
  - 1 Box of parafilm with scissors
- 24 25 mL fungal minimal media (FMM) agar plates with chloramphenicol (30 ug/mL)
- 24 25 mL fungal minimal media agar plates with 5 x 5 cm piece of UV sterilized mulch chloramphenicol (30 ug/mL)
- 24 25 mL fungal growth minimal media (GMM) agar plates with chloramphenicol (30 ug/mL)
- 16 25 mL M9 0 C minimal media (M9OC) agar plates with cycloheximide (50 ug/mL)
- 24 25 mL M9 0 C minimal media agar plates with 5 x 5 cm piece of UV sterilized mulch and cycloheximide (50 ug/mL)
- 16 25 mL M9 plus 0.2% glucose agar plates with cycloheximide (50 ug/mL)
- 40 PDA plus chloramphenicol (30 ug/mL) plates
- 40 1/10 X TSY plus cycloheximide (50 ug/ml) plates
- 40 1.5 mL 30% (w/v) glycerol in sterilized cryovials
- 30 1.5 mL 1/10X TSY agar slant cryovials
- 30 Sterilized Eppendorf tubes
  - 1 Dissecting microscope
Count colonies of mulch/soil dilution plates growing on TSY or PDA after incubating for five days at 20°C. Enter colony counts between 30-300 CFUs (others are labeled “TMTC” for too many to count, or “TFTC” too few to count) in Excel spreadsheet. Back calculate to estimate CFUs of fungi and bacteria per gram of mulch. The dilution plates are kept and used to streak individual colonies for the first BDM test (Fig. A1.7). The dilution plates containing well-separated colonies are used to streak 54 different isolates of both bacteria and fungi onto minimal media without carbon, minimal media with BDM as sole carbon source, and minimal media plus glucose agar plates in the first BDM test.

First BDM test. Isolates on the dilution plates exhibiting different morphology and color (Fig. A1.8A) are randomly chosen from each plate. The isolate is picked up with a sterilized flat-end toothpick (Fig. A1.8B), is transferred by streaking (10 mm) the tip of the single toothpick onto minimal media, then onto minimal media plus BDM (Fig. A1.8C), and finally onto minimal growth media. The same toothpick must be used for all three minimal media plates to ensure that the same cells/spores are being transferred.

Bacteria isolates are transferred onto M90C, M90C + BDM, and M9 + 0.2% glucose (see Section II). Bacteria isolates are streaked 15 to a plate (3 rows of 5) on M90C and M9 + 0.2% glucose, and 9 per plate (3 rows of 3) on M90C + BDM. Fungal isolates are transferred onto FMM, FMM + BDM, and GMM. Fungal isolates spread further and are streaked 9 to a plate (3 rows or 3) on all three plate types.

Agar dots (10 uL of either M90C or FMM top agar) are placed onto the mulch pieces to provide a water/nutrient (assumed minimal carbon) source for initial colonization (Fig. A1.6) because BDMs are hydrophobic and do not permit water to seep-through from the agar below.
BDM and other agar plates are prepared in advance (see media protocol). The toothpick containing the isolate is gently rubbed on the surface of the agar dot and then onto the mulch.

The plates are then inverted and incubated at 20°C in the dark for five days. It is important to check on fungal plates daily, because some isolates grow rapidly and can contaminate the entire plate. If rapid growth is seen, then the plates should be sealed with parafilm, and stored at 4°C to slow growth.

Minimal media agar plates are inspected for growth after the five day incubation period. If the bacterium grows on minimal media without carbon then you do not want to further test the isolate, because the bacterium is an autotroph. Autotrophs would be difficult to study and were beyond the scope of this study. Bacteria that do not grow on minimal media without carbon but do grow on BDM plates are the isolates of interest. Fungal isolates follow the same isolation pattern, so further test fungal isolates that do not grow on FMM but do grow on the BDMs. Fungal isolates that grow on FMM may be obtaining C from the agar that was used to solidify the medium.

Isolates that do grow on BDMs are streaked to single isolated colonies by picking up cells/spores from the BDM surface or agar dot with a sterilized flat-end toothpick and streaking onto TSY (bacteria) or PDA (fungi) plates. The toothpick is swiped across one plate quadrant multiple times (zig-zag fashion) then the toothpick is discarded and a new toothpick is used to spread cells/spores from the first quadrant to a second quadrant. This process is repeated until cells/spores have been spread to four quadrants. The toothpick should gently slide across the agar surface, avoid digging into the agar. Increase zig-zag spacing with swipes so that single colonies can be isolated with ease from later quadrants. Single colony plates are inverted, incubated at
20°C for five days in the dark, and monitored starting at day three to ensure isolate is growing and that the isolate is a pure culture.

**Second BDM test.** The second BDM test is required to confirm that the isolate is truly a potential biodegrader of the BDM mulch. Single colonies are isolated from the single colony streaked plates after incubating the inverted plates for five days at 20°C in the dark. A single colony is picked up with a flat-end toothpick and streaked onto a BDM plate. Again the toothpick is rolled on the agar dot that sits on top of the BDM piece then rubbed on the mulch. Two lawn plates are also made with single colonies, for subsequent storage. The lawn plates are for growing the isolate uniformly over nutrient media to maximize growth. Lawn plates are incubated along with the inoculated second BDM test plates at 20°C for five days. Bacterial lawn plates are made by streaking single colonies onto 1/10 X TSY with cycloheximide (50 ug/mL). Agar slants in cryovials are also inoculated with bacterial isolates and allowed to grow for short-term storage at 4°C. Fungal lawn plates are made by streaking a single colony onto PDA with chloramphenicol (30 ug/mL). In addition, an autoclaved filter paper disc is placed onto the agar of the fungal lawn plates, near the site of initial inoculum. When the paper is covered with mycelium/spores, it is removed with sterilized forceps, placed into a sterilized Eppendorf tube and air dried overnight (cap off), and then sealed and stored at 4°C. After five days of incubation at 20°C, the second BDM test plates are then rated using a dissecting microscope to visualize bacterial/fungal growth and characterize morphology. If the isolate grows on the BDM then the lawn plates are used to make permanent glycerol stocks of the isolate to be stored at -80°C for future testing.

**Preparation of isolates for long-term storage.** For bacterial isolates, glycerol stocks and agar slants are prepared for long-term storage, and for fungal isolates glycerol stocks,
colonized filter paper discs, and agar plugs are used for storage (Table A1.1). To prepare glycerol stocks, sterilized cotton swabs are wiped across the lawn plate, picking up cells/spores. After the whole plate has been swabbed, the swab is swirled into the glycerol to deposit cells/spores. One cryovial per lawn plate (i.e. two cryovials per isolate) is made, using a new swab for each plate. After the glycerol stock is made the cryovial is immediately mixed by shaking or vortexing, and transferred to a container with liquid N\(_2\). Use liquid N\(_2\) to flash freeze the sample because slow cooling allows for additional cellular damage. After all glycerol stocks are made, the pre-labeled cryovials are transferred from the liquid N\(_2\) to storage boxes and placed in a -80°C freezer. A pair of long forceps and mesh strainer can be used to pick up cryovials floating in the liquid N\(_2\). Agar slants are incubated at 20°C for five days and then stored at 4°C.

Storage of filter paper discs is done upon fungal colonization on the paper discs. The filter paper discs are removed from the agar surface with sterilized forceps and placed into a pre-labeled Eppendorf tube. The lid is left open and the filter paper is air dried in a closed biohazard safety cabinet with no airflow (to minimize cross-contamination) overnight. After eight hours the Eppendorf tube is closed and Parafilm is used to seal the top. The tube is then placed in a storage box and stored at 4°C. Lastly, an agar plug measuring approximately 0.5 x 0.5 mm is removed from the lawn plate with a sterilized razor (prior to cotton swabbing). The agar containing the isolate is then transferred to a pre-labeled Eppendorf tube, closed, sealed, then placed in a storage box and stored at 4°C.
**Fig. A1.1.** Steps to remove mulch pieces, sandwiched between soil inside a mesh bag. The bags were incubated in the soil during winter in 2010 until spring in 2011, following the growing season of a tomato crop. A, placement of mesh bag on clean plastic weigh boat B, carefully lifting mulch from soil after cutting mesh bag C, sterile scissors used to cut mulch into 1 x 1 cm pieces and weighed out to 0.05g D, a 15 ml culture tube with 9 ml of PBS broth and 0.05 g of mulch pieces E, sonicating culture tubes F, vortexing culture tubes and G, dilution of initial culture tube.

**Fig. A1.2.** Diagram showing the dilution process used to isolate microorganisms from biodegradable mulch pieces incubated in native soils.
Fig. A1.3. Dilution series A, from culture tubes with a pipette and B, from well plates with a multipipette.

Fig. A1.4. Diagram of improper and correct pipette transfers due to air bubbles or loose seal between the pipette and pipette tip, and how to transfer and mix solutions with pipette tips.

Well 1: slowly pull up solution and release down well wall

After well is mixed 10 times transfer 50 uL to next well and repeat mixing
**Fig. A1.5.** Final steps of dilution series **A**, transferring select solution onto selective media **B**, spreading solution with sterile glass tubes and **C**, counting colony forming units after incubation from a $5.0 \times 10^{-3}$ (left) and $5.0 \times 10^{-4}$ dilution (right).

**Fig. A1.6.** Steps demonstrating how to sterilize biodegradable mulches with UV light in preparation for placement onto minimal media.
Fig. A1.7. Set up of the first BDM test showing cultures of bacteria on minimal media used to determine carbon utilization of select isolates.

![Image of dilution plates containing 30-300 CFUs, MMOC minimal media, MMOC + BDM minimal media & BDM, MM + C growth minimal media]

One toothpick is used to pick up isolate and transfer to minimal media plates.

The isolate is streaked in the following order: MMOC, MMOC + BDM, and MM + C

Fig. A1.8. Procedure used to test select isolates for the utilization of carbon sources of biodegradable mulch film. A, agar plate showing distinct fungal colonies B, sterile toothpick used to pick up spores of one fungal colony and C, the same toothpick used to transfer spores onto agar dot and biodegradable mulch by streaking the tip of the toothpick.

![Images A, B, C showing agar plate, toothpick, toothpick on agar dot and biodegradable mulch]

Table A1.1. Long-term storage procedures used for pure cultures of fungal and bacterial isolates from the mulch samples.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Fungi</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Cryovials at -80°C</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cryovial agar slant at 4°C</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Agar plug at 4°C (Eppendorf tube)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Filter paper disc at 4°C (Eppendorf tube)</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
**APPENDIX B**

**SURVIVAL OF SCLEROTINIA SCLEROTIORUM IN SOIL**

**AS AFFECTED BY BIODEGRADABLE MULCHES**

*Sclerotinia sclerotiorum* (Lib.) de Bary, the causal agent of white mold and lettuce drop, is an important plant pathogen. This fungus can infect over 400 plant species (2) and remain viable in the soil, as sclerotia, for up to four years without a host (5). Sclerotia of *S. sclerotiorum* contain three layers: an outer, melanized rind, a middle cortex, and an inner loosely arranged medulla. The melanized resting structures aid in survival by reducing UV damage and microbial attack (7), and if damaged, rinds can be regenerated (3). However, the rind of the sclerotium does not limit water and nutrient flow.

Sclerotial exudates can be chemotactically sensed by mycoparasites such as *Coniothyrium minitans* Campbell, and stimulate growth of antagonists as has been demonstrated with sclerotial exudates of *S. minor* (4). Because biodegradable mulches used in agriculture are often incorporated into soil post-harvest, they could alter the survival of sclerotia by interrupting or enhancing antagonistic relationships between *S. sclerotiorum* and mycoparasites. This study evaluated survival of sclerotia as affected by soil incubation with select agricultural mulches in Skagit silt loam kept at field capacity.

The study was a four-by-four factorial study with four replications of four mulch treatments and four sampling times. Each experimental unit consisted of two 50 ml tripour beakers containing three sclerotia and 368 mulch pieces (total surface area of 92 cm$^2$) buried in field soil. Sclerotial inoculum was increased using a single isolate of *S. sclerotiorum*, Ss.Ls.2011, obtained from lettuce and cultured on half-strength potato dextrose agar (PDA) medium for approximately three to four weeks at room temperature under ambient light conditions. Mature,
melanized sclerotia, 2 x 4 mm to 3 x 6 mm, were collected from the PDA plates, air dried in a laminar flow hood over-night, and stored in a sterile petri dish in the dark at 20°C. The four mulches included: two commercially-available biodegradable mulches BioTelo Agri (BT, 0.02 mm, Dubois Agrinovation, Waterford, ON, CAN) and cellulose-based WeedGuardPlus (WG, 0.23 mm, Sunshine Paper Co. LLC, Aurora, CO); an experimental nonwoven spunbond poly(lactic) acid (PLA, 0.58 mm, NatureWorks LLC, Blair, NE) mulch; and, a non-mulch control. Mulch pieces, cut to 0.5 x 0.5 cm, were mixed by hand with field soil (moisture content at 22.75%) such that 1 g of soil contained seven mulch pieces. Soil was Skagit silt loam, located adjacent to where lettuce was grown in 2011, was collected with shovels down to 12.7 cm depth, and sieved (1 x 1 cm) into 22 liter buckets. This soil becomes congealed when wet. To increase soil moisture content, 1 ml of water was deposited to the base of a 50 ml tripour before adding the soil-mulch mix to allow water movement via capillary action. Within 30 minutes water reached the 10 to 15 ml level. After water was deposited, 25 ml of soil-mulch mix was added to the tripour. Three sclerotia were transferred with sterile forceps to the soil surface, and arranged equidistant to each other. An additional 25 ml of soil-mulch mix was gently poured over the sclerotia. Tripours were sealed with two layers of parafilm, then placed in sealed containers with moist paper towels, and incubated on a greenhouse bench at 21°C under shade cloth. After 11, 20, 33 and 40 weeks incubation, parafilm was removed and soil was air dried for two weeks. Sclerotia were recovered, and plated onto PDA with bromophenol blue (PDA-BPB) as reported by Steadman et al. (6) except that antibiotics were omitted in order to ascertain viability. Each sclerotium was surface disinfested prior to plating on the agar medium by submerging in 70% EtOH for four seconds and briefly passing through a flame. Plates were incubated at room temperature as above, and visually observed for color change and sclerotial formation over three
to 14 days. At each sampling time reference sclerotia, concurrently stored in the dark at room temperature, were also plated as above as a control on sclerotial viability. The experiment was repeated.

Percent viability across mulch treatments was not significantly different between the two experiments, and no interactions between the trials and mulch treatments were detected so the experimental data were pooled. Mulch treatments did not significantly affect viability of sclerotia at any sampling time, and percent viability did not follow any trend (Table 1). The viability of treatment sclerotia was lower, relative to reference sclerotia, through 33 weeks incubation: percent viability of the reference sclerotia was 86 to 100% for 20 weeks post inoculation, but then 14% at 40 weeks.

Although great effort was made to make the soil samples as homogenous as possible, there could have been areas where microbial activity was limited and antagonists were not present or near the sclerotia. It is also possible that mulch pieces, even when abundant, were still not sufficiently concentrated, incubated for a long enough period of time, or do not affect viability of sclerotia or activity of antagonists in native soil. Visual degradation of BT and SB-PLA pieces was rarely observed. Interestingly, even though WG, did not significantly affect viability of sclerotia, pieces of WG were always partially to fully deteriorated and blackened. WG breaks down readily since many soil microorganisms utilize cellulose as a carbon source (1).

Microorganisms with a saprophytic lifestyle may be more affected by biodegradable mulches than the necrotrophic fungus, S. sclerotiorum. The most common morphologically identified fungal genera that colonized the recovered sclerotia were Trichoderma, Penicillium, and Fusarium. Penicillium and Fusarium were often observed colonizing the same sclerotium
but growth on the culture medium was polarized suggesting that one may be parasitic while the other is opportunistic.

TABLE B.1. Percent viability of sclerotia of *Sclerotinia sclerotiorum* incubated in the dark with biodegradable mulches in Skagit silt loam soil for 11, 20, 33, 40 weeks at 21°C.

<table>
<thead>
<tr>
<th>Mulch</th>
<th>Initial</th>
<th>11 weeks</th>
<th>20 weeks</th>
<th>33 weeks</th>
<th>40 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mulch</td>
<td>---</td>
<td>25.00</td>
<td>15.63</td>
<td>13.54</td>
<td>15.62</td>
</tr>
<tr>
<td>BioTelo</td>
<td>---</td>
<td>9.38</td>
<td>27.08</td>
<td>14.58</td>
<td>11.46</td>
</tr>
<tr>
<td>Spunbond PLA-11</td>
<td>---</td>
<td>6.25</td>
<td>14.58</td>
<td>2.08</td>
<td>18.75</td>
</tr>
<tr>
<td>WeedGuardPlus</td>
<td>---</td>
<td>3.13</td>
<td>6.25</td>
<td>23.96</td>
<td>4.16</td>
</tr>
<tr>
<td><em>P value</em></td>
<td>---</td>
<td>0.1950</td>
<td>0.0687</td>
<td>0.6012</td>
<td>0.7558</td>
</tr>
</tbody>
</table>

**Trial**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Initial</th>
<th>11 weeks</th>
<th>20 weeks</th>
<th>33 weeks</th>
<th>40 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>---</td>
<td>7.81</td>
<td>13.54</td>
<td>12.50</td>
<td>11.46</td>
</tr>
<tr>
<td>2</td>
<td>---</td>
<td>14.06</td>
<td>18.23</td>
<td>14.58</td>
<td>13.54</td>
</tr>
<tr>
<td><em>P value</em></td>
<td>---</td>
<td>0.7555</td>
<td>0.7450</td>
<td>0.9033</td>
<td>0.5392</td>
</tr>
</tbody>
</table>

**Reference controls**

<table>
<thead>
<tr>
<th>Reference controls</th>
<th>Initial</th>
<th>11 weeks</th>
<th>20 weeks</th>
<th>33 weeks</th>
<th>40 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>94.44</td>
<td>85.71</td>
<td>100.00</td>
<td>57.14</td>
<td>14.29</td>
<td></td>
</tr>
</tbody>
</table>

*Percent viability calculated from total number sclerotia that germinated divided by total number of intact sclerotia recovered from soil as observed on half-strength potato dextrose agar (PDA) medium amended with bromophenol blue (BPB). The recovered sclerotia from soil treatments were dipped in 70% EtOH for 4 sec and then passed through a flame before the sclerotia were plated onto PDA-BPB. Sclerotia were considered viable if the agar plates turned yellow (indication of oxalic acid production) and secondary sclerotia were produced. Three sclerotia per sample were tested and each treatment had two samples per replicate with four replications per time sample; the experiment was repeated twice. No interaction was detected between trial and mulch, and therefore data from both experiments were pooled.

*Mulch treatments included a non-mulch control, two commercially-available mulches BioTelo (Dubois Agrinovation, Waterford, ON, CAN) and WeedGuardPlus (Sunshine Paper Co. LLC, Aurora, CO); and, an experimental nonwoven spunbond poly(lactic) acid mulch (NatureWorks LLC, Blair, NE). Each was cut into 0.5 x 0.5 cm pieces and mixed with native agricultural soil at the concentration of 7 mulch pieces per 1 g of soil.*

-Dashed lines (---) indicate viability not tested.

Sclerotia used in each trial came from the same *S. sclerotiorum* isolate recovered from lettuce and cultured on PDA for three to four weeks, the sclerotia were collected, air dried in a laminar flow hood overnight, and stored at 20°C in the dark. Sclerotia were either used in the mulch study or stored in the dark at room temperature to use as reference controls.

At each sampling time, seven reference sclerotia, concurrently stored in the dark at room temperature, were also plated as above as a control on sclerotial viability.
Literature cited:


Soil solarization for control of Verticillium was employed beginning 7 Jul in 2011 in replicated rows, 27 m long and 0.9 m wide, four high tunnel and four open field rows. Each high tunnel and open field row contained one control plot and five soil solarization plots each 4 m long and 0.9 m wide. The purpose of this preliminary study was to determine if solarization within a high tunnel, where air and soil temperatures are generally higher, might be more effective than solarization in the open field, and whether this practice could be explored as a soilborne disease control method in western Washington. In 2011, six lettuce cultivars were planted into raised beds that were previously planted with three strawberry cultivars grown in 2010. All of the strawberry cultivars, Albion, San Andreas, and Seascape, had developed Verticillium wilt caused by *V. dahliae* (data not shown). Subsequently, during the following 2011 lettuce growing season, five out of six lettuce cultivars planted (Adriana, Coastal Star, Ermosa, Green Star, and Jericho) also developed symptoms of Verticillium wilt caused by *V. tricorpus*. Soil samples to establish baseline populations of *Verticillium* were obtained from each row on 29 Jun 2011, after the lettuce roots had been removed, and included the plots in both solarized and non-solarized areas. Seven soil cores (2.0 cm in diameter x 20.3 cm deep) were removed from randomly selected planting holes in each former lettuce subplot. Core samples were obtained from each of the four replications, and were bulked, mixed by hand in plastic bags, and then air dried on paper boats for two weeks on a greenhouse head house bench. Drip tape (low flow, 1.6 cm diameter, 0.2 mm thick, 20 cm emitter spacing; John Deere Water, San Marcos, CA) and clear polyethylene (0.03 mm thick, 1.21 m wide, Robert Marvel Plastic Mulch
LLC, Annville PA) were manually laid on top of the beds on 6 Jul 2011. A single water saturation event occurred via drip irrigation, 1.9 cm was applied on 7 Jul. The soil was moistened so that the effects of soil solarization could be intensified as moisture will cause damage to microsclerotia when heat is added (4). A Hobo U-30 Weather Station (Onset Computer, Bourne, MA) was placed within one plastic-covered and one bare soil subplot in each of the high tunnel and open field main plots to record soil temperature at 20 cm below the soil surface at 15-minute intervals. The clear polyethylene film was removed on 6 September 2011 and additional soil samples were obtained from each plot on 9 September 2011 as described above. To assay populations of *Verticillium*, soil samples were ground with a clean mortar and pestle for 10 sec in a clockwise motion, and then one gram of each sample was plated onto 10 Petri dishes (100 x 15 mm) filled with semi-selective NP-10 agar media (1; 6). Salt shakers with eight 2 mm diameter holes and 8 mm spacing were used to evenly distribute the soil across the plates. Plates were incubated in the dark at 20°C for three weeks, soil was gently removed from the agar surface under a stream of running tap water, and *Verticillium* colonies counted at 20 X with a dissecting microscope. Colonies were categorized as *V. dahliae*, *V. tricorpus*, or *Verticillium* spp. based on colony morphology and pigmentation (2; 3). Each sample was counted three times and plates were stored at 4°C between each count.

The three month soil solarization reduced the total number of microsclerotia of *Verticillium* species (Fig. C1), including *V. tricorpus*, within the high tunnel treatment. Initial colony forming units per gram of soil (cfu/g) for all *Verticillium* spp. ranged from 0 to 16 cfu/g, and following soil solarization inoculum density ranges were reduced to 0 to 8 cfu/g. However, *V. dahliae* populations slightly increased when soil solarization occurred in high tunnels, but increased considerably from 0 to 8 cfu/g prior to solarization to 0 to 20 cfu/g after solarization in
open fields. *V. dahliae* can grow at 30°C whereas other Verticillium spp. such as *V. albo-atrum* are inhibited at 30°C (5). Bare soil averaged 19.8 and 17.3°C whereas solarized soil averaged 24.8 and 23.4°C in high tunnels and open fields, respectively (Fig. C2). The maximum soil temperature attained in high tunnel solarized plots was 32.2°C, while bare ground inside the tunnels reached 19.8°C. Soil temperatures above 30°C rarely occurred for a prolonged period of time (>4 h) inside the high tunnels and did not reach 30°C in the open field in either plastic-covered or bare ground.

Soil solarization in western Washington may not accumulate enough heat to reduce *Verticillium* populations in open fields but high tunnels were effective at reducing populations of some *Verticillium* species including *V. tricorpus*. Future studies should include an earlier application of clear polyethylene to determine if increased solarization time could also reduce *V. dahliae* populations. Additionally, biocontrol agents such as *Talaromyces flavus* could be applied after soil solarization to determine if an integrated pest management system would be beneficial for control of Verticillium wilt.
**Fig. C1:** Changes to *Verticillium* soil populations in high tunnel (HT) and open field (OF) productions systems after a soil solarization event, 7 Jul to 6 Sept 2011 near Mount Vernon, WA.

![Bar chart showing changes in CFU/g of soil for different *Verticillium* spp. in HT and OF systems after a soil solarization event.](chart1.png)

*Verticillium* spp.

**Fig. C2.** Comparisons of average daily soil temperatures between high tunnel (HT) and open field (OF) production systems recorded during a two-month soil solarization event from 7 July through 9 September 2011 near Mount Vernon, WA.

![Line graph showing soil temperatures over time for HT and OF systems.](chart2.png)
Literature cited


APPENDIX D

FIRST REPORT OF VERTICILLIUM WILT ON LETTUCE (Lactuca sativa L.)

IN WASHINGTON CAUSED BY Verticillium tricorpus I.

M. Powell, B. Gundersen, C. Miles, K. Coates, and D. Inglis Washington State University Mount Vernon NWREC, 16650 State Route 536 Mount Vernon, WA 98273-4768, and Washington State University Puyallup REC 2606 West Pioneer, Puyallup WA 98371-4900

Symptoms of Verticillium wilt were observed on lettuce harvested from consecutively planted experimental field plots in western Washington, 2010 to 2012. Leaves had v-shaped chlorotic lesions, were wilted, and discolored vascular tissue was noted in the crowns. Each year incidence of the disease increased from 0.2% to 1.9% to 14.4% between 2010 and 2012, although 194 out of the total 204 symptomatic plants were still considered marketable. Three isolates of Verticillium obtained from crown tissues were cultured on half-strength potato dextrose agar medium. The isolates produced a dark yellow pigment, black microsclerotia, white mycelia, tan chlamydomspores, and uni-septate conidia averaging 10.6 x 3.7 µm. The isolates were tentatively identified as V. tricorpus I. (1), and designated as Vt.Ls.2010, Vt.Ls.2011-1, and Vt.Ls.2011-2. Pathogenicity tests were completed in two greenhouse trials. In the first test, the three isolates were each inoculated onto eight seedlings of Coastal Star (CS) by clipping the roots and then dipping them into a suspension of 2.0 x 10^6 conidia/ml. In the second test, each isolate was inoculated onto five seedlings of CS with the suspension at 5.0 x 10^6 conidia/ml. The same cultivar and inoculation techniques were used for two sets of controls, five plants inoculated with an isolate of V. dahliae and five with sterile water. Plants were harvested eight to nine weeks post-inoculation and symptoms were visually rated. Vt.Ls.2010, Vt.Ls2011-1 and Vt.Ls2011-2, were pathogenic on 25, 13 and 13% of the CS plants in the first trial, and 40, 60
and 20% of the CS plants in the second trial. In comparison, V. dahliae was pathogenic on 25
and 40% of CS plants in the two trials, respectively. Plants inoculated with V. tricorpus had
some vascular discoloration and slight chlorosis on outer leaves while those inoculated with V.
dahliae had more severe symptoms noted by intensity and length of vascular discoloration. None
of the water control plants were symptomatic. V. tricorpus was re-isolated from inoculated
crown tissues and colony morphology was the same as that of the original isolates. Identification
of V. tricorpus was confirmed via ITS-PCR sequencing. (GenBank Accession numbers for V.
tricorpus isolates will be provided and descriptions given upon characterization of 2012
isolates). Currently, V. tricorpus is thought to be a weak pathogen of lettuce crops in the U.S. (2).
However, an isolate of V. tricorpus highly pathogenic to lettuce has recently been identified in
Japan (3). To our knowledge, this is a first report of Verticillium wilt caused by V. tricorpus in
Washington. Cropping lettuce continuously needs to be avoided due to the risk of increasing the
inoculum density of the pathogen in the soil.