

**Integrated management of bacterial spot (*Xanthomonas gardneri*) of
tomatoes (*Solanum lycopersicum* L.) in Southwestern Ontario**

by

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ABSTRACT

INTEGRATED MANAGEMENT OF BACTERIAL SPOT (*XANTHOMONAS GARDNERI*) OF TOMATOES (*SOLANUM LYCOPERSICUM* L.) IN SOUTHWESTERN ONTARIO

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Xanthomonas gardneri is the dominant bacterial spot pathogen affecting tomato production in Southwestern Ontario. There are no effective chemical controls for bacterial spot, so the potential for sanitation and host resistance management was investigated. Pathogen transmission in the plug trailer and transplanter were evaluated to gauge the potential impact of sanitation procedures, and differences in foliar and fruit symptoms of nine commercial cultivars were assessed to gauge existing host resistance. The pathogen moved 122 cm within the trailer, less when plug trays were dipped in water instead of irrigated. The transplanter transmitted the pathogen seedling to seedling but was not as impactful as the environment in field trials. There was no correlation between the incidence or severity of foliar and fruit symptoms in the commercial cultivars tested. Sanitation of the trailer and dip watering are recommended, as is inclusion of fruit symptom observations to select resistant cultivars.

DEDICATION

To everyone who helped me get here, those still here and not, against the voices that said, "no, you can't."

And to you reading this now, wondering if you too can do something similarly momentous; I did, so you absolutely can.

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LIST OF ABBREVIATIONS AND NOMENCLATURE

AIC – Akaike information criterion

AUDPC – area under the disease progress curve

BCMA – British Columbia Ministry of Agriculture

BSX – bacterial spot causing *Xanthomonad*

CAD – Canadian dollar

CGMMV – Cucumber green mottle mosaic virus

CKTM – Chang Kama Tween Medium

CMM – *Clavibacter michiganensis* subsp. *michiganensis* (bacterial canker of tomato)

DBH – days before harvest

DPC – disease progress curve

DTT – diseased transplanter treatment

DSI – disease severity index

HPC – hand-planted disease control

OMAFRA – Ontario Ministry of Agriculture and Rural Affairs

PDC – The University of Guelph's Pest Diagnostic Clinic

PCR – Polymerase chain reaction

RH – relative humidity

RTSA – rifampicin amended topic soy agar

sAUDPC – standardized area under the disease progress curve

TMV – Tomato mosaic virus

TSA – topic soy agar

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1 Literature Review

1.1 Introduction

Bacterial spot of tomatoes (*Solanum lycopersicum* L.) is caused by several species of *Xanthomonas*. The primary species affecting the Southwestern Ontario processing tomato crop is *Xanthomonas gardneri* (Cuppels et al. 2006; Abbasi et al. 2015).

Tomatoes are the second most popular vegetable in the world today based on sales and diversity, and a high-value crop for the region, with a 2017 gross farm value of nearly \$52 million CAD from 10,467 acres of production (Jenkins 1948; Foolad and Panthee 2012; OPVG 2018). Causes of potential yield loss are important to manage, in particular those like bacterial spot, for which growers currently have very limited management options (Jeffrey B. Jones et al. 2014; Abbasi et al. 2015; Trueman and LeBoeuf 2015; Roddy and Trueman 2018 Apr 19). Since bacterial spot can cause yield reductions of 60% the disease has a significant impact on the industry, and methods to manage it must be developed (LeBoeuf et al. 2009).

1.2 Tomato (*Solanum lycopersicum* L) and its cultivation

Tomatoes are one of more than 2300 species that belong to the genus *Solanum* in the family Solanaceae (Peralta et al. 2008). While the exact origins of the tomato (*S. lycopersicum*) are unknown, the Andean region of South America is home to its likely ancestor, the cherry tomato (*Solanum lycopersicum* var. *cerasiforme* L) (Heuvelink 2005). It is believed the cultivated species originated along the coast, in an area stretching from the equator as far south as the 30° latitude, but cultivation occurred in Mexico (J. Benton Jones 1999; Heuvelink 2005). The naming of the species also had

uncertainty, when in the 1750s Linnaeus and Phillip Miller described competing taxonomic identities, *Solanum lycopersicum* and *Lycopersicon esculentum*, respectively. This issue remained until modern genetic technology favoured Linnaeus' classification of *Solanum lycopersicum*, which is followed in this work (Foolad 2007a; Gerszberg et al. 2015).

Cultivated tomatoes produce more flowers and are self-compatible, important distinctions from wild relatives that rely on cross-pollination (J. Benton Jones 1999). Tomatoes require relatively warm temperatures for production; optimum temperatures are 21-24°C and 18-21°C for day and night, respectively (J. Benton Jones 1999). Flowering or fruit defects may occur at temperatures below 16°C, and above 35°C, and at temperatures below 10°C, growth is completely inhibited (J. Benton Jones 1999). Heat units alone can predict plant development since the plant is insensitive to photoperiod (Gould 1992; Heuvelink 2005).

Five hundred years of population bottlenecks and inbreeding reduced the genetic variation of today's cultivated tomato (Heuvelink 2005; Gerszberg et al. 2015). Small differences in phenotype are exploited for different uses, as is the case with determinant and indeterminate types. Indeterminate plants grow in a single vine by removing offshoots from the stem, which can be maintained for months in the field or greenhouses (J. Benton Jones 1999). These cultivars have fruit with higher juice content and are ideal for the fresh market (Iijima et al. 2016). Determinant types are grown in fields, their stems and offshoots terminating in flowers for more uniform fruit production for processing (Gould 1992; J. Benton Jones 1999). Processing fruit has less

juice, more pulp and must pass an Agtron colour rating for the load to be considered acceptable (Rick 1978; OPVG 2006; Consortium 2012).

For processors in Ontario, maximizing the season is important due to climate restrictions, so plants are started in the greenhouse and transplanted beginning in early May (Biggs and Stewart 1965; Gould 1992; Heuvelink 2005). This requires thousands of seedlings to pass through the transplanting machine per hectare, and each plant must be handled by a worker. After planting, some manual weed control may be done depending on the success of the pre-plant herbicide program. Sandy loam is often the preferred soil because it is fit to plant sooner, facilitating pre-plant herbicide application and the early planting the crop requires (Gould 1992). Well-drained soil is an asset to keep the farm operation moving during spring planting and fall mechanical harvest, as meeting processor deadlines is paramount. Transplanting and harvest dates are coordinated to align with processing operations, so conditions are not always optimal, and transplant shock is common (Biggs and Stewart 1965; Gould 1992). Transplanting in twin rows is also standard practice for Ontario growers, leading to high plant populations in fields.

Time to maturity varies by cultivar, but growers may use heat units, days post-flowering or rely on scouts to determine fruit development (Gould 1992; Heuvelink 2005). The processor may schedule the harvest by having growers spray their fields with ethephon, a compound that breaks down into ethylene to trigger ripening. This makes the harvest more uniform and predictable (Gould 1992; Farag and Palta 1993). Processors dictate the number of loads received from a field per day, and all harvest is

completed using a mechanical harvester, which cuts the vine and picks up everything on the soil surface (Gould 1992). Vines are shaken, so the fruit breaks free and can be sorted. Workers then sort the fruit, removing any with defects or discolouration, with mechanical eyes assisting with colour sorting (Gould 1992).

In general, a mature tomato fruit is a red berry, but cultivars do exist with different carotenoids and, therefore, different colours (Gould 1992; J. Benton Jones 1999; Khachik et al. 2002). Lycopene is usually the most abundant carotenoid, making up 83% of the polyene compounds that produce the colour of the fruit (Gould 1992). It is the sum of the different carotenoids, including alpha, beta, gamma and delta-carotene, in addition to lycopene and carotenol that ultimately produce the fruit colour of a cultivar (Gould 1992). Separately, the number of locules the cultivar possesses determines its fruit size. For processing tomatoes the fruit usually possess only two locules, making a smaller fruit, while fresh market beefsteak cultivars may have more than six (J. Benton Jones 1999).

Once pollination has occurred, the ovary rapidly changes, and the development of fruit begins (Ren et al. 2011). If fruit set is successful, the fruit begins to mature, ideally reaching size in a few weeks. The mature green stage is indicated by fruit reaching 80% of their expected size, dropping surface hairs, and the start of locule maturity, which begins once the seeds are physiologically mature (Giuliano et al. 1993; Taiz et al. 2015). This fruit stage is important because in the processing industry ethephon applied to mature green fruit will induce ripening, but immature green fruit are unresponsive.

As lycopene and polygalacturonase replace starches in the fruit, the tomato enters the breaker stage, and red pigment becomes visible. Once the fruit in a field reaches approximately 90% red, they are considered red-ripe and harvested for processing (Gould 1992; J. Benton Jones 1999). Processors usually test loads using mechanical colour tests, such as the Agtron, and other visual assessments for fruit defects or disease, to determine the grade of the load and, therefore, its value (Biggs and Stewart 1965; Gould 1992; J. Benton Jones 1999). The Agtron, for example, uses two wavelengths to measure the internal and external colour (Gould 1992). Another quality measure is a soluble solids (Brix) test, a proxy to measure the sugar content of the tomatoes by light refraction (Gould 1992). To be acceptable, the fruit must also fall within size constraints, commonly determined by its ability to pass through shaking grates of the desired size (Gould 1992; J. Benton Jones 1999).

1.3 Bacterial spot causing Xanthomonads

All *Xanthomonas* species are Gram-negative, rod-shaped bacteria that produce a pale-yellow colony on artificial media. Classification within the *Xanthomonas* genus changes with new evidence and techniques due to the bacteria's few distinguishable characteristics (Boch and Bonas 2010). For example, secretion systems can be too ubiquitous within the genus for species separation (Schornack et al. 2008; Boch and Bonas 2010).

The organism now classified as *X. gardneri* was originally isolated by Šutić in Yugoslavia, and called *Pseudomonas gardneri* (Šutić 1957). In 2004 an effort to clarify the genus was published by Jones et al. (2004). This most recent accepted

reclassification incorporated modern evidence with realigned nomenclature to more closely reflect the original names (Jones et al. 2004). This reclassification elevated *X. gardneri* from no official taxonomic status to a separate species, as it shows 70% or less homology with other *Xanthomonas* species causing bacterial spot on tomato and pepper (Jones et al. 2004). *X. gardneri* is also referred to as a bacterial spot causing Xanthomonad (BSX) Group D, where Group A is *Xanthomonas euvesicatoria*, Group B is *X. vesicatoria*, and Group C is *Xanthomonas perforans* (Jones et al. 2004). The group based classification has followed previous reclassifications throughout the 20th century (Doidge 1921; Gardner and Kendrick 1923; Dowson 1939; Young et al. 1978; Hildebrand et al. 1990; Vauterin et al. 1990; Palleroni et al. 1993; Bouzar et al. 1994; Stall et al. 1994; Bouzar et al. 1996; Jones et al. 2000). The historical classification of the Xanthomonads is of relevance because species names associated with bacterial spot of tomato shift in the scientific literature as classification has evolved, and it is not always clear which species was studied using current species classification. For example, in the 1990's *X. campestris* pv. *vesicatoria* strains affecting tomato were subdivided into races T1 (now *X. euvesicatoria*), T2 (now *X. vesicatoria*) and T3 (now *X. perforans*), based on which tomato cultivars elicited a hypersensitivity response (HR) (Jones et al. 1998; Astua-Monge et al. 2000: 4; Scott et al. 2015). The tested cultivars were 'Hawaii 7998', 'Hawaii 7981' and 'Bonny Best.' The Hawaii cultivars were the first cultivars to show some resistance to BSX, while 'Bonny Best' was a common susceptible standard (Jones et al. 1998). T1 strains illicit a HR response from 'Hawaii 7998', while T3 illicit a HR response from 'Hawaii 7981'. Strains which caused no HR

from either Hawaii cultivar or 'Bonny Best' were designated T2. Under this nomenclature, *X. gardneri* did not have an official taxonomy (Jones et al. 2004; Scott et al. 2015). As of November 2018, there is another push to have *X. gardneri* reclassified, this time as a heterotypic synonym of the artichoke pathogen *Xanthomonas cynarae* (*X. cynarae* pv. *gardneri*), so the issue of a proper name is far from settled (Timilsina et al. 2019). But for the remainder of this review the accepted 2004 reclassification taxonomy by Jones will be followed as closely as possible and when not specific to *X. gardneri*.

Xanthomonas gardneri is a significant pathogen of field-grown tomatoes in many production areas including Ontario, Canada; Ohio, Michigan and Pennsylvania, United States of America; Malaysia and Brazil (Quezado-Duval et al. 2005; Cuppels et al. 2006; Kim et al. 2010; Ma et al. 2011; Abbasi et al. 2015; Rashid et al. 2015; Trueman and LeBoeuf 2015). In Ontario, bacterial spot-causing Xanthomonads with copper tolerance are present (Abbasi et al. 2015). A recent Ontario survey found bacterial spot symptoms caused mostly by both *X. gardneri* (36%) and *X. perforans* (26%), while *X. vesicatoria* and *X. euvesicatoria* respectively made up just 1 and 2% of the population (Abbasi et al. 2015). The remaining 35% of the strains collected by Abbasi et al. (2015) could not be placed into a single species.

The primary source of bacterial spot causing Xanthomonads is thought to be infested seeds and transplants imported from endemic areas (Stall et al. 2009; Giovanardi et al. 2018). *Xanthomonas campestris* pv. *vesicatoria* survives over 100 days on untreated tomato seed (Bashan et al. 1982). The same study also found *X. c.* pv. *vesicatoria* survives for over ten months on pepper seeds buried in soil in Israel,

once a stable population of 10^4 colony forming units (CFU) g^{-1} is reached (Bashan et al. 1982). To measure survival on seeds, Bashan et al. (1982) stored infested seed in a hermetically sealed glass box, which was buried in a field in Israel for 1.5 years. During the experiment, soil temperature measured as low as $0.3^{\circ}C$ and as high as $42.2^{\circ}C$. Electron microscopy revealed that *X. c. pv. vesicatoria* can colonize pepper fruit without developing symptoms and the bacteria was successfully isolated from the ovaries of fruit, indicating seeds are a source of primary inoculum (Bashan et al. 1982; Bashan and Okon 1985). However, research on *X. euvesicatoria* showed peppers produced by infected plants only carried 100 CFU g^{-1} , which was insufficient to produce an outbreak (Giovanardi et al. 2018).

Field residue is a potential source of inoculum in warm climates. For example, in Israel and the Middle East, *X. c. pv. vesicatoria* was recovered from the rhizosphere after 18 months (Bashan et al. 1982). However, there is no evidence of overwintering in Southwestern Ontario soils or nearby areas like Ohio (Cuppels et al. 2008; Jeffrey B. Jones et al. 2014; Ma 2015). In Ohio, contaminated plant material was buried in three different soil types and *X. gardneri* was not recovered from any of them after six to twelve months (Ma 2015). This is in contrast to earlier work done in Florida showing limited survivability of *X. c. pv. vesicatoria* outside the host (Bashan et al. 1982; Jones et al. 1986). *Xanthomonas gardneri* is, however, known to survive as an epiphyte on seed and remains epiphytic on developing cotyledon leaves in tomato (Stall et al. 2009; Kelley and Boyhan 2017).

Infection of the plant after the epiphytic stage occurs via natural openings, like stomata, or through wounds (Leben 1981; Bashan and Okon 1985; Jeffrey B. Jones et al. 2014). Symptomatic plants typically have population of 10^5 CFU g^{-1} of *X. c. vesicatoria* compared to asymptomatic plants with 10^2 CFU g^{-1} (Jones et al. 1991). Bacterial spot causing Xanthomonads are favoured by heat, humidity and rain, with *X. gardneri* having an optimal temperature of 24 to 30°C, and spreading effectively in water (Gardner and Kendrick 1923; Tartier and Pitblado 1994; J. B. Jones et al. 2014). Other than movement in water, BSX are transferred among plants by animals, plastic or metal tools, and air movement (Bashan 1986; McInnes et al. 1988). Since BSX have an epiphytic phase, this transfer could occur without immediate infection and development of symptoms. Mud on implements also carried the same level of bacteria as the seemingly clean surface, highlighting the difficulty in keeping implements and personnel pathogen free (Bashan 1986). If the bacteria are well established epiphytically, then favourable conditions encourage infection and symptom development, and symptoms can appear suddenly after conducive weather events.

The mechanism of BSX entry via stomata is not well understood. For the bacterial speck pathogen *Pseudomonas syringae* pv. *tomato*, this is an active process whereby the pathogen excretes effectors, which cause the stomata to open, and an immune response to the same effectors triggers closure of stomata by the host (Melotto et al. 2006). For BSX, effects on gene regulation have only been studied for *X. euvesicatoria* (Zhang et al. 2009). Zhang *et al.* (2009) used green fluorescent protein to show that the type III secretion systems were upregulated before the invasion of the

host and not simply once inside (Bonas et al. 2000; Zhang et al. 2009). The upregulation of secretion systems before invasion suggests active entry into the host and additional effects of the secreted compounds (Alfano and Collmer 2004; Wichmann et al. 2013). Unfortunately, GFP tagging alone is not sufficient for this investigation due to potential to affect the tagged proteins' function. Extensive biochemical monitoring methods, similar to those employed by Melotto et al. (2006), would be required to confirm the active or inactive nature of *X. gardneri* infection of a host.

Once foliar infection has occurred, BSX multiply within host tissues. For *X. vesicatoria*, this occurs predominantly in the leaf veins and away from the stomata (Sharon et al. 1982). Upon invasion, host phytohormones act in a similar fashion to a mammalian innate immune system, whereby pathogen-associated molecular patterns interact with specialized pathogen recognition receptor proteins and phytohormones produced by the host, resulting in a chemical signal relay to produce the appropriate defense proteins (Pieterse et al. 2009; Toruño et al. 2016). Xanthomonads, in turn, are equipped with a variety of effectors which affect these phytohormones and their pathways (Boch and Bonas 2010). In addition, some type three secretion system effectors activate transcription of host genes, which increase host susceptibility, in addition to host-specific toxins, ultimately resulting in symptom development (Friesen et al. 2008; Boch and Bonas 2010). The infection of host fruit is not as well understood as infection of the foliage. Without the presence of stomata, it is believed that trichomes are the main point of entry, and those are lost as the fruit matures (Getz 1983; Gudesblat et al. 2009a; Gudesblat et al. 2009b).

Bacterial spot symptoms include brown-black lesions up to 5 mm in diameter on tomato leaves (Figure 1-1A, D and E) (Tartier and Pitblado 1994; Jeffrey B. Jones et al. 2014). Infected blossoms rapidly become necrotic (Figure 1-1E), while lesions on fruit appear scabby, water-soaked and the skin may crack in a star shape (Tartier and Pitblado 1994; Potnis et al. 2015). A fruit lesion may grow to a diameter of 5 to 8 mm (Figure 1-1B). Losses occur when yield and quality are reduced. As the plant defoliates, photosynthetic potential is reduced, while sunscald risk on fruit increases (Koike et al. 2006; J. B. Jones et al. 2014). The loss of photosynthetic potential reduces yield, while quality is reduced by both sunscald and skin lesions, which cause skin tags when peeled for processing (Figure 1-1C).

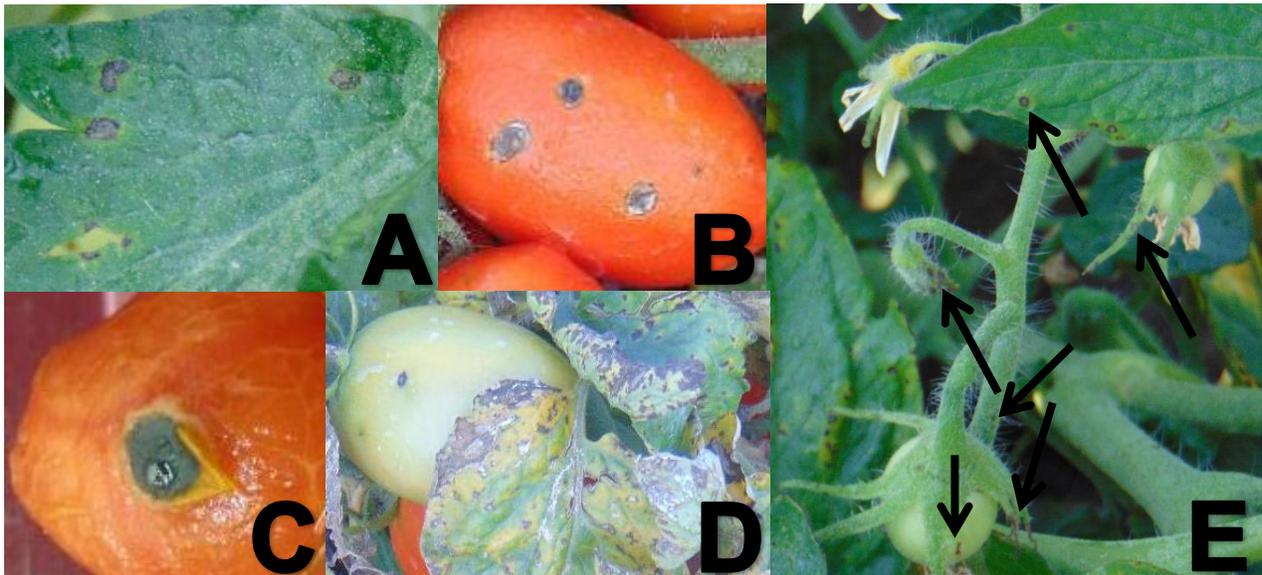


Figure 1-1. Examples of damage caused by bacterial spot of tomato

(A) Leaf spots caused by *X. gardneri* (B) Fruit lesions caused by *X. gardneri* (C) Skin tag damage caused by *X. gardneri*, image courtesy of Dr. C. Trueman (D) Defoliating damage of coalescing spots caused by *X. gardneri* (E) Tomato spots caused by *X. gardneri*, on all host surface tissue.

1.4 Current management strategies

Current management of bacterial spot is challenging. Survival of *X. gardneri* in the Ontario landscape, including on soil and plant residue, is limited (Cuppels et al. 2008; Yates et al. 2011; Ma 2015), therefore common cultural controls such as tillage and crop rotation have limited or no effect. Current management strategies focus on seed quality, chemical, and biological control, with limited availability of host resistance.

1.4.1 Seed treatments

The use of clean seed is a standard recommendation, but detection and treatment of infested seed is difficult. Seed treatments have been suggested to manage the disease at its source, but issues persist with treatments like chemical or hot water baths, such as cost and potential effect on germination rates (Goode and Sasser 1980; Jones 1991; Tartier and Pitblado 1994). Hot water and chemical bath treatments have been tested, as they work well when used to treat whole seed lots for fungal or viral diseases, respectively (Goode and Sasser 1980; Dhanvantari and Brown 1993; Lewis Ivey and Miller 2005). In the 1980s hot water treatments (56°C) were observed to decrease germination by 10% when seeds were soaked for five minutes more than the 25 minute recommendation (Goode and Sasser 1980). More recently, seed germination was found to be slightly slower with hot water treated seed, but growth was not overly impaired, and pathogen presence was reduced (Ma et al. 2011). It is still very difficult to completely eliminate a bacterial pathogen from seed without damaging the seed germ, and even very small numbers of Xanthomonads on seed could be enough to support an outbreak in the field (Potnis et al. 2015).

The potential to damage the seed, and risks associated with reduced germination, slowed the implementation of hypochlorite treatments (Dhanvantari 1989). The careful use of chlorine bleach or hot water treatments are now being used to reduce pathogen populations on seed (Lewis Ivey and Miller 2005; Dick and Dick 2014; Lewis Ivey et al. 2015). There are also accredited testing standards, administered by the International Seed Federation, to test for the presence of pathogens, both viral and bacterial, including *Xanthomonas* species, on sample seed lots to see if treatments are effective or warranted (International Seed Federation 2017). But there are still no registered seed treatment recommendations for bacterial spot from the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA 2018a).

1.4.2 Chemical and biological control

Due to limited options and convenience, copper foliar sprays have been the dominant pest management strategy for tomato bacterial spot for several decades (Zevenhuizen et al. 1979). In Canada, copper octanoate and copper hydroxide are labelled for management of bacterial spot (OMAFRA 2018a). Copper octanoate (Cueva) is registered for bacterial spot control in greenhouse and field production, as are many copper hydroxide products (Coppercide WP, Kocide 2000, Parasol WG) (Kocide LLC 2017 Jan 10; Nufarm Agriculture INC 2017 Jan 10; W. Neudorff GMBH KG 2017 Jan 10). Unfortunately, shortly after copper solutions were noted as bactericidal, resistant strains of *X. c. pv. vesicatoria* were identified, as well as plasmids carrying copper resistance genes (Marco and Stall 1983; Bender et al. 1990). Implementation of programs using copper in combination with alternative biological or chemical controls,

as well as tank mixing copper with the the fungicide mancozeb have been explored for some time as a means to extend the usefulness of copper. Copper-mancozeb mixtures, plant activators and most recently small particles like 3-Indolylacetonitrile have been shown to better manage copper insensitive strains of *X. campestris* pv. *vesicatoria* in Florida, where copper alone became completely ineffective (Marco and Stall 1983; Louws et al. 2001; Liu et al. 2019).

Non-copper products registered for bacterial spot on field tomatoes in Ontario include kasugamycin (Kasumin®), *Bacillus subtilus* QST 713 (Serenade Opti®), and extract of *Reynoutria sachalinensis* (Regalia Maxx®) (Su 2012; Abbasi and Weselowski 2015; Arysta LifeSciences 2016; CropScience 2017; OMAFRA 2018b; Certis 2019a; Certis 2019b). Kasugamycin, *B. subtilus* and *R. sachalinensis* extract are listed for suppression only on OMAFRA pest control guidelines (OMAFRA 2018b). *Bacillus subtilus* acts by producing biofilms on plant roots and tissues and is capable of producing a surfactant to form an additional barrier to infection (Bais et al. 2004). *Reynoutria sachalinensis*, unlike other biocontrols, is not used as a whole organism but as a concentrated extract and induces host resistance by increasing the plants production of phytoalexins, phenolic compounds and other defence related proteins (Daayf et al. 1997; Su 2012). In New York and North Carolina, *R. sachalinensis* extract applied without copper was shown to be ineffective against *X. campestris* pv. *vesicatoria* (New York) and *X. perforans* (North Carolina) at both high and low doses (Lange et al. 2018; Meadows and Sharpe 2018). In Florida, kasugamycin tank mixes showed no effect against *X. perforans*, but the plant activator acibenzolar-S-methyl

applied in low concentrations weekly or high concentrations biweekly was able to reduce disease without harming yield (Vallad and Huang 2011a; Vallad and Huang 2011b). *Bacillus subtilis* strain QST 713, used in combination with copper hydroxide applications was also effective in Florida (Roberts et al. 2008). The fungicide quinoxyfen (Quintec®) has also been tested in Florida for efficacy against bacterial spot (Vallad and Huang 2011c; Vallad et al. 2013 Sep 4). However, in North Carolina, quinoxyfen was not different from the untreated control (Meadows and Sharpe 2018).

Many of these copper alternatives, including copper hydroxide with mancozeb, *Bacillus subtilis* QST 713, *Reynoutria sachalinensis*, kasugamycin, and various combinations of each, were tested against *X. gardneri* at the University of Guelph, Ridgetown Campus, over three years (Trueman 2015a). There was inconsistent disease suppression from non-copper products and treatment combinations that did not contain copper hydroxide (Trueman 2015b). For example, when the extract of *R. sachalinensis* was used in combination with alternating copper applications, disease suppression was observed against a copper sensitive isolate of *X. gardneri*; however, as with other non-copper treatments, it was not different from the nontreated control when applied without copper (Trueman 2015a). Acibenzolar-S-methyl tank mixed with copper hydroxide was the only treatment that consistently reduced plant disease, similar to results against copper-resistant strains of bacterial spot, causing Xanthomonads in Florida (Huang et al. 2011). However, as of 2015 acibenzolar-S-methyl is no longer available to growers in Ontario, and copper foliar sprays alone are not recommended due to resistance (Filotas 2015; Trueman and LeBoeuf 2015).

The use of antibiotics has been avoided due to concerns the bacteria could develop resistance and possibly spread that resistance to other microbes (Stall et al. 2009). This is exemplified by streptomycin, one antibiotic which has been used to manage bacterial spot (Ritchie and Dittapongpitch 1991). The antibiotic is registered for use on tomatoes in the United States, but not in Canada (EPA 1992; USDA 2006; Health Canada 2018; National Center for Biotechnology Information 2019). Plasmids have been found in the pathogen population conferring resistance to streptomycin (Minsavage et al. 1990; National Center for Biotechnology Information 2019). Therefore the useful life of the antibiotic against bacterial spot is limited, though resistance can vary by geography (Minsavage et al. 1990; Ritchie and Dittapongpitch 1991; Pernezny et al. 1995) .

Bacteriophages, or 'phages' are viruses that target and infect bacteria, sometimes with such specificity as to only affect a specific subspecies (Acheson 2011). This specificity means they do not affect non-target microflora and are safe for use and consumption in agricultural settings (Flaherty et al. 2000). Against *Xanthomonas campestris* pv. *pruni*, bacterial spot of peaches, a phage selected for efficacy on many bacteria isolates proved highly effective when applied prior to inoculation (Zaccardelli et al. 1992). Because bacteriophage are so specific in efficacy, selecting for the proper phage may be difficult in a field setting, but an additional issue is the rapid dessication of bacteriophage. In peach orchards, while 92% efficacy was obtained with preemptive phage application, the product residue was only viable for five days, which makes it difficult to use as a preventative (Zaccardelli et al. 1992). Several different phages have

been selected for use against tomato BSX and different product formulations tested to increase residual activity (Flaherty et al. 2000; Balogh et al. 2003). Beyond phage specificity and residual, there are still issues with phage replication predictability and even application timing, making it difficult to use them in practice (Obradovic et al. 2004; Balogh et al. 2018 Sep 18).

One additional avenue of interest has been plant growth-promoting rhizobacteria, applied as foliar or seed treatments, to induce systemic resistance in the host (Ji et al. 2006). *Pseudomonas fluorescences* 89B-61 and a biocontrol strain of *Pseudomonas syringae* Cit7 are whole bacterium biocontrols tested against bacterial spot *X. campestris* pv. *vesicatoria* and *X. vesicatoria* in Alabama and Florida (Ji et al. 2006). *Pseudomonas fluorescence* 89B-61 has been investigated due to its production of toxic compounds, while *P. syringae* Cit7's mode of action is less well understood (Hamdan et al. 1991; Duffy and Défago 1999; Byrne et al. 2005). Although both biocontrols were found to be able to reduce disease when applied individually, there was no significant additive effect when applied together. Additionally, the biocontrols were not as reliable from one trial to the next compared to copper-mancozeb, which offered the same level of efficacy (Ji et al. 2006).

1.4.3 Host resistance

There is an important distinction between host tolerance to bacterial disease and resistance. Resistance is defined by a host that kills the pathogen or prevents it from causing infection (McCarville and Ayres 2018), while tolerance indicates the existence of a synergy between the host's metabolism and immunity that allows it to remain

healthy while infected, having a neutral impact on the pathogen (McCarville and Ayres 2018). Host resistance is an important part of integrated pest management strategies for plant diseases.

One of the challenges with creating a bacterial spot resistant host is the ability of the pathogen populations to shift over time to different related species within a region (Abbasi et al. 2015). Breeding of tomatoes for resistance to many diseases has been aided by recent advances in molecular markers to identify and track resistance genes in breeding lines, which was recently summarized and reviewed (Foolad and Panthee 2012; Sharma and Bhattarai 2019). The pursuit of a bacterial spot resistant tomato may rely on the use of genetic editing, and a resistant, productive host remains a long-term goal (Tai et al. 1999; Wang et al. 2011; Brooks et al. 2014). Currently, two major suppliers of commercial processing tomato seed in Ontario, Heinz Seeds and Tomato Solutions, offer a few cultivars with “intermediate” or “noticed” resistance to bacterial spot (Dick and Dick 2018; Heinz 2019). Unfortunately neither seed supplier gives specific data about which causative organism(s) the resistance was tested against or whether differences were observed in number or severity of lesions on foliage or fruit (Dick and Dick 2018; Heinz 2019). Such modest resistance is usually due to the lack of specific (qualitative or vertical) resistance (*R*) genes that can be used to combat the disease. Unlike qualitative resistance genes such as *Ve*, *Pto*, *Asc*, which confer full resistance to *Verticillium dahliae* race 1, *Pseudomonas syringae* pv. *tomato* race 0, and *Alternaria alternata* f. sp. *lycopersici* respectively in tomatoes, BSX are managed

through quantitative (horizontal) genes (Heinz 2019). This form of resistance is more difficult to breed for as many genes must be tracked together.

Breeding for quantitative resistance is ongoing using molecular markers (Hutton, Scott, Yang, et al. 2010; Scott et al. 2015). Quantitative genes work synergistically, creating a more resilient defence for the host, and remaining effective longer than single genes conferring qualitative resistance genes (Kunwar et al. 2018). Resistance is most often bred by starting with a known resistant parent line, then inbred and offspring lines are screened for resistance (Somodi et al. 1994; Francis and Miller 2005). Two breeding lines, 'Ohio 9834' and 'Ohio 9816', with at least partial foliar resistance to *Xanthomonas campestris* pv. *vesicatoria* race T1 (likely *X. euvesicatoria* under Jones 2004), have been produced with identified mutations in the hypersensitivity response, known as *Rx3*, that might be exploitable for further adaptation into commercial cultivars (Francis and Miller 2005; Yang et al. 2005; Yang and Francis 2005). The cultivar 'Hawaii 7998' was the first tomato cultivar to show resistance to bacterial spot (Whalen et al. 1993). Unfortunately, the number of non-dominant and independent genes required for the resistance hindered breeding, and over time the spot species 'Hawaii 7998' was resistant to, *X. euvesicatoria* T1, was replaced with *X. perforans* T3, a species to which 'Hawaii 7998' had no resistance, highlighting the difficulty of this task (Whalen et al. 1993; Wang et al. 2011; Sharma and Bhattarai 2019).

Tracking multiple genes through a breeding program is difficult, but tools like molecular markers, coupled with CRISPR gene editing, now make the task easier (Hutton, Scott, Yang, et al. 2010; Brooks et al. 2014; Sharma and Bhattarai 2019). Such

markers were necessary for the development of 'Ohio 9834' and 'Ohio 9816' because of the difficulty of maintaining multiple genes in a breeding line. Unfortunately, the incorporation of resistance genes from related wild Solanacea have had negative effects on quality traits such as fruit size, limiting this source of resistance genes (Foolad and Panthee 2012; Aleksandrova et al. 2014). There have been some recent breakthroughs in breeding for resistance to another bacterial disease, bacterial wilt (*Ralstonia solanacearum*), where resistance genes were incorporated using selective breeding with progeny derived from Hawaii 7998 without losing plant size or flavour qualities (Scott et al. 2009).

For most evaluations of resistance only foliar data are compared and presented, as fruit can be very labourious to assess (Pervaiz A. Abbasi et al. 2002; Al-Dahmani et al. 2003; Obradovic et al. 2004; Francis and Miller 2005; Yang and Francis 2005; Ji et al. 2006). However, this may not always be sufficient to judge resistance. There are anecdotal reports from industry in Ontario that the fruit of some cultivars are more susceptible to infection by *X. gardneri* or that fruit lesions on some cultivars are more severe, regardless of the level of foliar infection (Dr. C. Trueman, pers. comm.), but this has not been published in the literature. For example, composted yard waste was observed to increase disease severity on foliage in conventionally grown tomatoes, but during the year with the highest disease incidence, the compost treatment had the lowest bacterial spot incidence (P. A. Abbasi et al. 2002). In the first year of the study, foliar disease severity was 104% lower in the no compost treatment than the high rate of compost treatment, but at the same time incidence on fruit in the high rate compost

treatment was 50% lower than the no compost treatment, showing an inverse relationship (P. A. Abbasi et al. 2002). The second year of study, the no compost treated controls had lower bacterial spot incidence on foliage and fruit than the compost treatments, but the maximum percent infection found on fruit was low at less than 5%.

In another experiment comparing tomatoes treated with acibenzolar-S-methyl to those treated using a bacteriophage, the foliar disease control was again not followed by improved quality or quantity yield in treated plants (Obradovic et al. 2004). When disease incidence was high, foliar disease control treatments varied by up to 36%, but no difference in yield was found, while low disease incidence showed foliar treatments varied by 39% and yield deviated by 28% across treatments. During low disease incidence, the highest yield did not occur in the same treatment as the lowest foliar incidence. This shows a lack of correlation between foliar health and increased yield, in terms of quantity or quality.

Separate from the long breeding process, there have been advances in transgenic resistance whereby the integration of the Arabidopsis ELONGATION FACTOR TU RECEPTOR gene and the pepper gene *Bs2* into a Florida tomato variety susceptible to *X. perforans* (Kunwar et al. 2018). But long term effectiveness of this combination is unknown, and a tomato created to be resistant to the dominant bacterial spot pathogen in Florida may not be as effective against another. BSX populations can be so diverse that differences within a state can cause separate populations to develop (Egel et al. 2018). Another challenge for the application of transgenic tomatoes for disease management is a lack of consumer acceptance. American, Japanese and

Norwegian consumers reporting a willingness to pay premiums up to 62%, 40% and 69% respectively for non-transgenic products over transgenic equivalents (Chern and Rickertsen 2001). A more recent study on American Millennials still found attitudes towards transgenic products were negative, despite knowledge of positive effects such as drought resistance (Linnhoff et al. 2014). The Flavr Savr tomato was the first genetically modified organism to be declared safe and sold in the 1990s, but failed because of poor consumer opinion of transgenic foodstuffs (Fraboni 2017; Komives and Kiraly 2017).

1.5 Developing future management strategies

A lack of effective management methods for bacterial spot suggests that alternative BSX management strategies are needed to address the management gap. In Southwestern Ontario, seedlings are produced in greenhouses for approximately first six weeks before being transplanted in the field (Gould 1992). This is an ideal environment for an epiphytic population to spread. Similarly, when seedlings are transported in canvas-covered plug trailers from the greenhouse to the field, and then transplanted, the environment is ideal for *X. gardneri* spread because seedlings are placed in close proximity and each seedling is touched by workers and passes through transplanting equipment (Kelley and Boyhan 2017). Despite advances in disease and defence in experimental breeding lines, there are currently no commercial cultivars available to growers that carry reliable resistance to *X. gardneri*. Differences in foliar and fruit resistance among commercial cultivars in Ontario may exist but this has not been explored in research trials. Therefore, an investigation of ways to reduce early

season dissemination of *X. gardneri* by seasonal activities, as well as any differences in cultivar resistance between fruit and foliage is warranted.

1.5.1 Seasonal activities and sanitation

Sanitation is a common strategy in pest management and is applied regularly in greenhouse and animal production systems (OMAFRA 2014; Baysal-Gurel et al. 2015; Li et al. 2015; Miller et al. 2015). For example, mastitis management in dairy cattle, which is caused by several environmental bacteria including Staphylococci, Streptococci and *Escherichia coli*, is dependent on sanitation of milking equipment and a germicidal dip applied to the teats after milking (Reinemann et al. 2003; Sharif and Muhammad 2009). In plant agriculture, equipment may be in contact with plants both in the greenhouse and the field, but sanitation guidelines generally exist only for greenhouse operations. Canadian guidelines on greenhouse sanitation are general, and meant to address the cleanliness of the greenhouse itself as well as equipment and people that come in contact with plants (OMAFRA 2009; BCMA 2018). The British Columbia Ministry of Agriculture (BCMA) offers a list of recommended disinfectants including; 70% ethanol, peroxide, quaternary ammonium compound products (KleenGrow®), alcohol based hand sanitizers for personnel (Purell®), and potassium peroxymonosulfate (Virkon®) for sanitation of footwear (BCMA 2018). KleenGrow® is the only product recommended for sanitation when plants are present, as it does not require rinsing (BCMA 2018). One product that can be applied directly to tomatoes or working surfaces is OxiDate 2.0 (hydrogen peroxide and peroxyacetic acid), which has both antifungal and bacteriacidal propensities (BioSafe Systems 2018). OxiDate 2.0

must be rinsed off of metal surfaces due to corrosion and is not registered for use against bacterial spot specifically. The BCMA also recommends commercial vehicles that are used for greenhouse work be professionally steam cleaned before returning to the greenhouse (BCMA 2018).

Some greenhouse implements like pruners also have self sanitizing functions, and can be loaded with disinfectant that is automatically dispensed (BCMA 2018). These still require the correct sanitizer to be selected by the user. Any obstructing organic matter also needs to be removed from the implement and sufficient exposure time allowed for the disinfectant to be effective before the equipment is used again. This is essential, as work done with bacterial canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis* ((Smith) Davis, Gillaspies, Vidaver & Harris)) (CMM) has shown that bacteria can be transferred to up to 22 plants through contaminated tools, while only one infected plant in 10,000 is required to cause an outbreak (Chang et al. 1991; Sharabani et al. 2013; Giovanardi et al. 2018).

Although efficacy data supporting the benefits of sanitizing greenhouses and field equipment is not available for most tomato pathogens, good hygiene practices are always recommended. The effectiveness of sanitation protocols with products as simple as bleach solution has been shown against viral tomato pathogens in greenhouses even when applied to plants (Li et al. 2015). Li et al. (2015) found that, unchallenged, tomato mosaic virus (TMV) killed all plants of cultivar 'VTV' that it infected, but when a 10% hypochlorite or 2% potassium peroxydisulfate (Virkon®) solution was applied to the inoculated plants less than 10% of plants became symptomatic. In California olive

orchards, the novel quaternary ammonium compound QAC-2 effectively reduced the population of *Pseudomonas savastanoi* pv. *savastanoi* on contaminated orchard implements by 99.9%, from 1×10^5 to 100 CFU/mL, when the pH of the solution was 6 to 9. QAC-2 remained effective in the presence of organic matter contaminants, which is important if implements cannot be thoroughly cleaned (Nguyen et al. 2017).

Relatively simple and effective sanitation procedures are the most likely to be implemented by operators, and addressing the potential spread of a pathogen early should limit epidemics. If phytotoxicity from sanitation is minimal, and the procedure is effective, then the extra cost may be warranted for diseases like bacterial spot (Goode and Sasser 1980; Trueman and LeBoeuf 2015). One example of sanitation recommendations is in Nigeria, for the management of bacterial wilt disease caused by *Ralstonia solacearum* (Fajinmi and Fajinmi 2010). The dominant causal agent in that region is of race 3, biovar 2, an aggressive isolate that must be strictly managed (Adebayo and Ekpo 2005). This pathogen presents a similar problem to *X. gardneri*, as it is widely distributed, there is inconsistent chemical management, and minimal host resistance. Phytosanitation management of *R. solacearum* is the main recommendation to reduce pathogen movement from one area to another, including removal of all weeds or plant residue from fields, disinfecting equipment between fields, controlling flood water flow and using only groundwater for irrigation, although the benefits of these practices have yet to be quantified (Fajinmi and Fajinmi 2010).

1.5.2 Host resistance in foliage and fruit

Foliar symptoms of BSX are usually observed before fruit symptoms in the field, and healthy foliage is often associated with positive plant health (P. A. Abbasi et al. 2002; Scott et al. 2011). However, there are anecdotal reports from industry that the fruit of some processing tomato cultivars is more susceptible to infection by *X. gardneri* than foliage compared to other cultivars, or that fruit lesions on some cultivars are more severe, regardless of the level of foliar infection (C. Trueman, pers. comm.). This has not been reported in the literature, in fact, tissue specific symptom distinctions with this disease are only rarely reported in the literature, foliar and yield metrics are much more common.

Potential causes of a potential disconnect in resistance between foliar and other tissue types are poorly described, but they have been noted in tomatoes for BSX, and in potatoes for *P. infestans* (Roer and Toxopeus 1961; Peters et al. 1999; Abbasi and Weselowski 2015). It is not uncommon to find foliage and fruit data presented in papers evaluating pest control products or methods, though reasons for any differences in observations between foliage and fruit go unexplained (Roer and Toxopeus 1961; Peters et al. 1999; Abbasi and Weselowski 2015). Even when test products appear to reduce foliar damage in efficacy trials, their effectiveness on fruit, the marketable tissue, is sometimes lower than that seen on the foliage (Pervaiz A. Abbasi et al. 2002). Assessing fruit is much more labourious than foliage, so it is likely that other examples exist, but go unnoticed or unreported.

Phytophthora infestans infection of potato presents one of a few examples where differences in symptoms are relatively well reported, even on host cultivars with varying degrees of resistance to the pathogens (Roer and Toxopeus 1961; Peters et al. 1999). *P. infestans* infects the foliage and tuber of potato in a temporally separated infection cycle. Potato tubers can only become infected after the pathogen infects and reproduces on the above ground vegetation, requiring new inoculum to be washed down to the tuber to infect it. Roer and Toxopeus (1961) reported differences in *R* genes in potato against *P. infestans* in 1960s, identifying that for the three *R* genes known at the time each had very different effects in leaves than in tuber flesh, with *R*₂ and *R*₃ not affecting tuber resistance at all (Roer and Toxopeus 1961).

Peters et al. (1999) tested many *P. infestans* isolates against potato cultivars with variability in resistance, finding that surface necrosis and lesion depth varied widely depending on cultivar. When challenged with many different pathogen isolates, cultivar 'Russett Burbank' had greater surface necrosis of the tuber but lower tuber penetration compared to 'Kennebec', and 'Shepody'. A similar study showed that, as with different tuber tissues, foliage and tuber disease severity also differed (Inglis et al. 1996). Most cultivars had relatively low tuber rot, but 'Shepody' and 'Norkotah', the cultivars that had greater rot than the thirteen other cultivars in the study did not show greater foliar damage. 'Shepody' had lower or equivalent AUDPC than seven cultivars, including 'Russet Burbank', while 'Norkotah' was lower than or equal to 'Shepody', 'Goldrush', 'Superior' and 'Hilite' when disease incidence was high. Peters et al. (1999) noted their results represented exceptions to the perceived rule in potato breeding that robust

foliage meant a resistant plant, though an exploitable mechanism was not found (Peters et al. 1999). Some of this disconnect has since been attributed to differences in the induction cascade of major resistance genes to *P. infestans*, rather than different genes (Orłowska et al. 2012). For example, Orłowska et al. (2012) noted that the resistant cultivar 'Sarpo Mira', rated high for foliar resistance to *P. infestans*, but lower for tuber resistance (Orłowska et al. 2012). It was not the major resistance genes alone that provided resistance to *P. infestans*, but rather seven genes, transcribed earlier in the immune response cascade, which were not present in other cultivars where resistance in foliage and tubers was similar (Orłowska et al. 2012).

While tomatoes lack major resistance genes to BSX, it is possible that differences in tissue responses between the foliage and the fruit exist as observed in potatoes for *P. infestans*. Another possibility could be the existence of proteins akin to thionins in different tomato cultivars causing differences in symptom development. Thionins are small defense peptides often associated with ovary and seed tissue that help protect against seed borne pathogens. Some thionins have been identified in peppers (*Capsicum annuum*), which are members of the same plant family as tomato (Bohlmann and A 1994; Iwai et al. 2002; Taveira et al. 2016).

Tomato fruit are believed to be susceptible to infection by *X. gardneri* until they reach three centimeters in diameter (Getz 1983). Susceptibility is believed to be lost at this point because trichomes fall off as fruit mature beyond this size, eliminating the entry point for the bacteria, as is the case with *P. syringae* pv. *tomato* (Getz 1983).

Unfortunately, it has been difficult to document infection of fruit by Xanthomonads so

this mode of entry remains unconfirmed, with infection through the flowers being another possibility (Bashan and Okon 1985). This is a stark contrast to foliar infection, where stomata are documented as the primary entry point (Melotto et al. 2006; Gudesblat et al. 2009b). Many Xanthomonads have been found to produce protein effectors that actively open the stomata, but little is known of effectors for fruit infection (Melotto et al. 2006; Gudesblat et al. 2009b; Boch and Bonas 2010).

Potential differences in the infection mechanisms of foliage and fruit by *X. gardneri*, observations of differences in the efficacy of crop protection tools for BSX on foliage and fruit, and differential resistance of foliage and tubers of potato, a close relative of tomato, to *P. infestans*, suggest the possibility of differences in resistance in tomato foliage and fruit to BSX. If true, cultivar evaluation may require fruit evaluation, which would increase the labour required significantly. When cultivars are developed by breeders, it is the foliage that is generally assessed, with the Horsfall-Barrett scale being commonly used (Scott et al. 2003; Hutton, Scott, and Jones 2010). To justify the development and implementation of additional screening protocols for breeders, a better understanding of foliar and fruit resistance is needed.

1.6 Research rationale

In 2000 an outbreak of bacterial spot lead to the first reports of *X. gardneri* in North America (Cuppels et al. 2006). Previously, copper tolerant Xanthomonads had not been detected in Ontario, and the established populations could be managed by copper-based foliar sprays (Cuppels et al. 2006; Abbasi et al. 2015). Without resistance management these practices lead to a high prevalence of copper-resistant *X. gardneri*

(Abbasi et al. 2015; Trueman and LeBoeuf 2015). This practice is no longer recommended in Ontario, and an effective replacement approach. A remaining option is to integrate as many small changes as possible to exclude the pathogen from production fields, such as sanitation prior to field establishment, to limit the reintroduction of *X. gardneri* in Southwestern Ontario each year. Additionally, in the pursuit of resistant cultivars disease free foliage is a major criteria, but this may not be sufficient to produce resistant plants (Scott et al. 2003; Hutton, Scott, and Jones 2010). This suspicion comes from anecdotal reports from industry that the fruit and foliage of some cultivars are not equally susceptible to infection by *X. gardneri* (C. Trueman, pers. comm.). Therefore the investigation into both the potential for sanitation and differences in localized host resistance on different plant parts is necessary to better manage this disease.

1.7 Objectives

The objective of this project was to investigate the potential benefits of improved sanitation, and host resistance in foliage and fruit, to identify alternative bacterial spot management strategies. The first objective was to assess disease movement through (a) irrigation in transplant trailers and (b) during transplanting, and the second objective was to evaluate foliar and fruit tolerance to bacterial spot when (a) foliage is inoculated, and (b) when flowers and fruit are inoculated.

1.8 Hypotheses

It was hypothesized that:

1. *X. gardneri* is transmitted plant to plant via trailer and irrigation activities.
2. *X. gardneri* is transmitted plant to plant via transplanting activities.
3. Fruit symptoms will show more variability between cultivars than foliar symptoms.

2 Potential for transmission of *Xanthomonas gardneri* during tomato transport and transplanting

2.1 Abstract

Xanthomonas gardneri is the dominant causal agent of bacterial spot affecting Ontario field tomato production today. This pathogen is tolerant of copper-based chemical controls, with no effective alternatives available, causing major production issues. The impact of any transmission of the pathogen at or just before transplanting is unknown, so *X. gardneri* transmission in irrigated trailers and planting equipment was studied in controlled environments. In one study, symptomatic seedlings were placed at the top of a simulated plug trailer with healthy seedlings placed 30.5, 61.0, 91.5, and 122 cm below to assess pathogen movement potential in four replicated trials with different irrigation treatments. Irrigation treatments were applied, and trays incubated overnight inside the trailer. Disease incidence (percent seedlings with symptoms) 14 days after irrigation in the top to bottom (3.9%) and bottom to top (5.4%) irrigation treatments were greater than the dipped treatment (0.4%). In diseased treatments, symptoms were found at all measured distances below the inoculation point. The second study saw wet or dry symptomatic seedlings pass through a transplanter before healthy seedlings, which were collected, incubated, and pathogen populations enumerated after 14 days. Epiphytic *X. gardneri* was found on healthy seedlings from all diseased treatments. A third study evaluated the impact of the machine induced transmission in the field, with healthy plants planted after symptomatic ones to assess the environmental influence on pathogen movement. Results indicate *X. gardneri*

transmission is possible in plug trailers and during transplanting, but environmental factors predominantly influence movement in the field.

2.2 Introduction

Bacterial spot of tomatoes (*Solanum lycopersicum* L.) is an important production issue for the processing tomato industry of southwestern Ontario (Abbasi et al. 2015). Tomatoes are an important and economically valuable crop in Ontario, with a gross farm value approaching \$52 million in 2017 (OPVG 2018). Bacterial spot can cause yield reductions of 60% as foliar lesions made by the bacteria cause leaf defoliation, and infected flowers become necrotic (Tartier and Pitblado 1994; LeBoeuf et al. 2009; Potnis et al. 2015). Beyond potential yield loss, lesions can develop on fruit, decreasing the value of the remaining crop (LeBoeuf et al. 2009). Copper-based bactericides were the standard treatment for the management of bacterial spot in the past, but widespread insensitivity to copper is now reported in Ontario (Kuflu and Cuppels 1997; Abbasi et al. 2015). There are no highly effective alternatives available for chemical or biological control (Trueman 2015b; Trueman and LeBoeuf 2015).

There are many related species of *Xanthomonas* that cause bacterial spot of tomato and pepper crops (Jones et al. 2004). In Southwestern Ontario *Xanthomonas gardneri* (Šutić) is the most common cause of the disease, (Kuflu and Cuppels 1997; Jones et al. 2004; Abbasi et al. 2015; C. Trueman, pers. comm.). The organism now classified as *X. gardneri* was originally isolated by Šutić in Yugoslavia and reported under the name *Pseudomonas gardneri* (Šutić 1957). *X. gardneri* has now been reported in the United States, specifically in Pennsylvania, Michigan and Ohio, Brazil,

and Malaysia, but not yet in major tomato production areas such as California and Florida (Quezado-Duval et al. 2004; Kim et al. 2010; Ma et al. 2011; Rashid et al. 2015). Without effective host resistance and chemical or biological controls, reducing the spread of the bacteria in the early season through more diligent sanitation remains an unexplored option. Tomatoes in southwestern Ontario are started as transplants and then transplanted outdoors when conditions are favourable, which allows the plants to be started early, in a controlled environment, where they can establish with uniformity (Biggs and Stewart 1965; Gould 1992; Heuvelink 2005). Part of the planting cycle requires seedlings to be moved from the greenhouse to the field by loading dozens of trays into canvas-covered trailers, then transplanting the seedlings. This requires thousands of plants to be moved placed in canvas trailers for transportation to the field, sometimes sitting overnight before they can be planted. Plants are usually watered immediately before being placed in the trailer and again in trailer if held for long periods, which creates an ideal setting for disease transmission. Seedlings are then individually fed into the transplanting apparatus by hand. Over 7,600 plants pass through the transplanting machine per hectare, with each plant handled by a worker at least once (OMAFRA 2010). Unfortunately, in the industry regular cleaning and sanitation of trailers and transplanting equipment, or of workers hands, is uncommon.

These procedures produce conditions that are ideal for the spread of bacterial pathogens. For example, movement of *X. perforans* (Jones et al. 2005) during overhead irrigation in greenhouse tomatoes was demonstrated to occur at a rate of 2.54 to 6.10 cm/day (Abrahamian 2017; Vallad 2019). Bacterial canker of tomatoes is another

example of a bacterial disease that can be spread during the greenhouse phase (Kawaguchi et al. 2010). In Japanese greenhouses, where plants are watered from below, symptom monitoring indicated that plant residue and contaminated tools were also major contributors to disease spread, affecting at least four plants post contact with an infected one (Kawaguchi et al. 2010). The viral pathogen cucumber green mottle mosaic virus (CGMMV) has been similarly shown to transmit to at least nine plants through hand pruning (Li et al. 2016). For CMM populations established on the host, copper bactericides mixed with streptomycin or acibenzolar-*S*-methyl were able to significantly reduce pathogen populations compared to an inoculated control (Werner et al. 2002; Hausbeck 2017). However, acibenzolar-*S*-methyl is not available to Canadian growers (Werner et al. 2002; Filotas 2015).

Studies on the influence of plug tray transportation and field equipment contaminated with *X. gardneri* on pathogen dissemination and bacterial spot symptom development are lacking. As such, the importance of these activities as a transmission pathway for *X. gardneri* was investigated using three sets of trials. In the first set of trials, the influence of irrigation techniques within transplant trailers on bacterial spot development was evaluated. In the second and third set of trials, the influence of contaminated transplanting equipment on a) the development of bacterial spot symptoms and *X. gardneri* populations in controlled conditions and b) the development of bacterial spot symptoms under field conditions were evaluated. The objective of this work was to determine if transport and transplanting practices are important transmission pathways for *X. gardneri*.

2.3 Materials and methods

Three different trials evaluated the ability of *X. gardneri* to spread during the early season, through transport and transplanting activities. A trial to simulate trailer dissemination was set up in the greenhouse at the University of Guelph, Ridgetown Campus, Ridgetown Ontario (42°26'55.9" N 81°53'05.3" W) and replicated four times. In each replication of the trial pathogen presence, identified by percent seedlings displaying symptoms, and symptom distribution throughout the trailer were observed by inspection of individual seedlings in each of the four trays in a plot. An indoor transplanter dissemination trial was completed three times in a growth chamber, while an outdoor transplanter dissemination trial was completed once per year, 2016-2018, at Ridgetown Campus. The indoor trial evaluated bacterial spot symptom development and pathogen populations from plants that had passed through the transplanter following symptomatic plants, while for the outdoor dissemination trial, the number of days until symptoms developed on plants at various distances from the known infection point was measured.

2.3.1 Trailer dissemination

Trailer dissemination trials were set up in greenhouse compartment #7 at the University of Guelph, Ridgetown Campus. Mock trailers were represented by metal shelving units (122 cm x 61 cm x 198 cm), with the particleboard shelves replaced by five wire mesh shelves (Figure 2-1). The wire shelves allowed water movement and facilitated shelf spacing of 30.5 cm, the same distance separating shelves in

commercial plug trailers. A plastic tarp was wrapped around each unit to simulate the canvas used to cover commercial plug trailers.

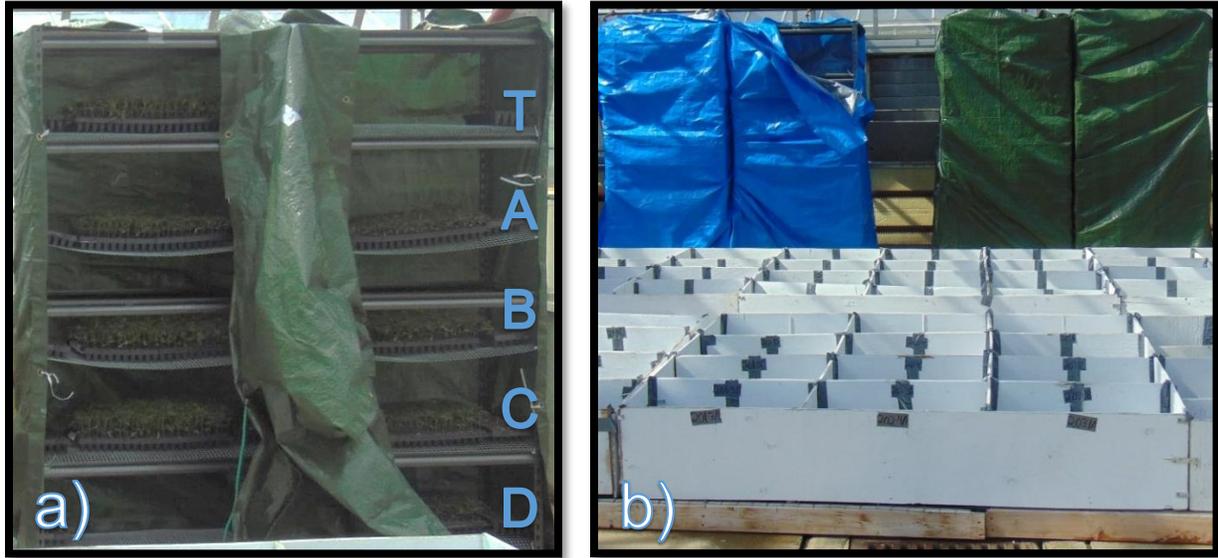


Figure 2-1 Mock trailer and incubation grid design.

a) Tarp-covered treatment mock trailers used in the trailer transmission trials, University of Guelph, Ridgetown Campus greenhouse, 2017-2018 in two trials per year. Symptomatic trays were placed on top (T) shelf and effect of different irrigation treatments on percent incidence of bacterial spot was measured after water treatments and overnight incubation were applied on trays 30.5 cm (A), 61 cm (B), 91.5 cm (C) and 122 cm (D) below the inoculated level.

b) Painted plywood isolation grid system for tray incubation. Trays A – D were placed in wooden isolation barriers for a 14-day incubation period to allow symptom development.

Six-week-old tomato seedlings 'H5108' (HeinzSeed, H.J. Heinz Company, Chicago, Illinois) grown in 288-cell trays and produced at a local commercial greenhouse were used in all trials. The trial was arranged as a randomized complete block design with split plots. Each mock trailer containing five trays of seedlings acted as an experimental unit, while the trays were separated from the inoculum source by a set distance, creating a split-plot. The tray placed at the top of the trailer served as the inoculum source, with the first healthy tray placed 30.5cm below the inoculated tray (shelf A). The second, third and fourth seedling trays were placed 61.0 (shelf B), 91.5 (shelf C) and 122.0 cm (shelf D) below the inoculated tray, respectively. In the first trial of 2018, an error occurred during transportation resulted in 30-40% of seedlings in four trays being damaged by heat. These trays were used as bottom trays (122.0cm from top) as spare trays were not available. Four distinct watering techniques were tested per replicate, so that differences in symptom development relative to both distance from inoculum source and watering technique could be assessed. Environmental conditions in the greenhouse compartments were monitored using wireless HOBO MX2301 Temperature/RH Data Logger systems (Hoskin Scientific, Burnaby, BC), and the data are presented in Appendix 2.

Tomato seedlings with symptoms of bacterial spot were used as an inoculum source. To produce these seedlings for field trials, four-week-old tomato seedlings 'H5108' were inoculated with *X. gardneri* DC00T7A an isolate originally collected by Dr. D. Cuppels at Agriculture and Agri-Food Canada in London Ontario, Canada in 2000,

and re-isolated through pathogenicity testing in 2017 at the University of Guelph Ridgetown Campus, or with *X. gardneri* DC00T7A-RDC-7 a rifampicin-resistant mutant of DC00T7A isolated at Ridgetown Campus, University of Guelph in 2018 for indoor trials. At least three days before inoculation bacteria were plated on TSA (DC00T7A) or RTSA (RDC-7), grown at room temperature and used to create a bacterial solution of 1×10^7 CFU/mL for inoculation of treatment trays. Trays were sprayed with the bacterial solution and placed under benches which had been covered with painter's plastic to create a humid environment for overnight incubation. After incubation, seedlings were placed on a misting bench in a separate compartment from the main trial in the Ridgetown Campus greenhouse. Misting occurred every 15 minutes between 7:30 am and 5:00 pm.

Irrigation treatments were applied over two days with replications one and two on Day 1 and replications three and four on Day 2. On the first day, healthy plants were obtained from the commercial greenhouse and loaded into the mock trailers. Symptomatic trays were placed into the mock trailers at 2:00 pm. Irrigation treatments were applied using a hose with a Dramm 750PL, yellow breaker nozzle (Dramm Corporation, Manitowoc, WI) either from top to bottom, the bottom to the top, or by dipping the trays in container of water 94 cm long by 60 cm wide and 10 cm deep. The container held water 6 cm deep, enough to submerge the plugs of the trays without wetting the foliage. The healthy control was irrigated by watering with a hose from the bottom of the trailer to the top. Trays watered by nozzle were watered for 3 to 4 seconds per shelf, using a back and forth motion, while those watered by dipping were dipped for

60 seconds, starting with the top tray. The nozzle and first 30 cm of hose were sanitized with 70% alcohol, the dip water changed, and container cleaned with Purell® Advanced Hand Sanitizer Gel (70% ethanol v/v) between each applicable treatment (GOJO Industries, Akron, OH, U.S.A) between plots. Once treatment application was complete, tarps were wrapped around the mock trailer and plants incubated until 7:00 pm, when the irrigation treatments were repeated.

At 8:00 am the next morning, the lower four trays were removed from the mock trailers and placed in an interlocking wooden grid, beginning with the bottom tray. The grid system consisted of plywood barriers 60 cm long, 30 cm wide and 40 cm high, painted with Beauti-Tone Signature Series Exterior Latex Flat White paint (Home Hardware Stores Ltd, Burford ON) , and corners sealed with caulking and layers of duct tape, which was placed on wire and mesh tomato pallets to improve draining and air movement. This grid was used to separate plots and prevent inter-plot interference. Inoculum trays were discarded, and the mock trailers were cleaned by spraying with water, removing plant debris, and disinfected by spraying with a solution containing 10% Dettol (4.8% Chloroxylenol) (Reckitt Benckiser Group plc (RB), Slough, Berkshire, England). Mock trailers were tarped while additional plants were collected from the local greenhouse, after two hours the sanitizer was rinsed from the mock trailers and air-dried. Once dry, trailers were reloaded for treatment of replicates three and four at 2:00 pm.

Incubation of treatments in the wooden grid occurred for 14 days, except for the first trial in 2017, which was incubated for eight days due to plants overgrowing the

barriers and abundant disease development. Trays were watered regularly using overhead irrigation. Lesions were initially diagnosed by comparison to a visual guide made from examples over previous years (Appendix 1). Foliar spots present as watery, grey, circular lesions, with a necrotic center, the tissue surrounding the lesion may yellow producing a halo effect around the center. In addition, to help confirm the symptoms developed were solely the result of the applied treatment, one additional tray of plants per replicate were placed in a separate greenhouse compartment and incubated on a bench for 14 days so any latent disease from the initial greenhouse could be identified through these 'holdback' plants. As before, environmental conditions in this greenhouse compartment was monitored using a wireless HOBO MX2301 Temperature/RH Data Logger and presented in Appendix 2. At the end of the incubation period, every plant in each tray of holdbacks was inspected for symptoms of bacterial spot.

2.3.2 Indoor dissemination

Assessments were completed over two days to ensure an equal incubation time for all replications. The total number of seedlings per tray and the number of seedlings with symptoms was used to calculate the incidence of seedlings with bacterial spot. If lesions were present, at least one lesion per plot visually identified as bacterial spot was sampled to confirm it was *Xanthomonas* spp. In addition, suspicious lesions that only somewhat fit the visual criteria for bacterial spot were sampled. This was done by plating on topic soy agar (TSA) and Chang Kama Tween Medium (CKTM), a semi selective media for Xanthomonads, to ensure correct identification (Sijam et al. 1991;

Cuppels et al. 2006). Depending on colony morphology one X-like plate and any non-X-like plates per trial were sent to the Pest Diagnostic Clinic (PDC) at the University of Guelph, Laboratory Services (Guelph, ON) for molecular identification, using Amplified Fragment Length Polymorphism primers originally developed by Koenraadt *et al.* (2009), to differentiate BSX (Koenraadt et al. 2009). X-like colonies were defined as pale-yellow colonies that grew as circular, raised mucoid mounds on artificial media, and when grown specifically on CKTM the colonies were also typically surrounded by a white crystalline halo.

To assess dissemination of the pathogen via contact with transplanting equipment, an indoor dissemination trial was completed in a growth room at the University of Guelph Ridgetown Campus twice per year. Tomatoes of cultivar 'H5108' were produced by a local greenhouse until they were six weeks old, as described in section 2.3.1, then brought to the Ridgetown Campus greenhouse before being treated.

Symptomatic seedlings were produced as described in section 2.3.1; all inoculated seedlings were inoculated with the rifampicin-resistant mutant *X. gardneri* RDC-7. After incubation in 2018, seedlings were placed on a misting bench in the Ridgetown Campus greenhouse. Misting on the bench occurred every 15 minutes between 7:30 am and 5:00 pm. In 2019 the misting bench was unavailable, so seedlings were misted by hand until runoff using a hand pump sprayer at 8:00 am, 10:00 am, 12:00 pm, 3:00 pm and 5:00 pm daily until symptoms developed.

The trial was arranged in a randomized complete block design with four replications per treatment. A custom two-row tomato transplanter (RJ Equipment, Blenheim, ON) was used to apply treatments. There were three treatments per trial, which included a healthy control with wet foliage, symptomatic plants with wet foliage, and symptomatic plants with dry foliage. In symptomatic treatments, six symptomatic plants were passed through the transplanter, followed by 52 visually healthy plants. Every symptomatic plant was collected by catching it after it fell through the transplanter, but before hitting the ground, and individually bagged. Following the symptomatic plants, every fifth seedling was bagged and collected. Between plots, the transplanter equipment was rinsed with tap water, sterilized with 70% alcohol and dried using an air compressor. Because the equipment was a two-row planter plots ran on alternating sides of the machine, allowing more time for disinfection and drying. The hands of the plant catcher were sterilized using Purell® Advanced Hand Sanitizer Gel (GOJO Industries, Akron, OH, U.S.A) after each plant was bagged. The remaining plants from the healthy trays after treatment application were placed in another growth room and monitored for the development of bacterial spot symptoms for 14 days.

Bagged plants were incubated overnight in the growth room set to 24°C, and a 16-hour photoperiod. The following day all plants were individually potted, organized by plot, and plots separated using plastic dividers to prevent cross-contamination. Within plots, plants were arranged in plant order through the transplanter. Plants were incubated in the growth room for 14 days and assessed for bacterial spot symptoms as described in section 2.3.1. For each trial the temperature and percent relative humidity

(%RH) were measured hourly by a HOBO MX2301A (ONSET, Bourne, MA, USA). Environmental data for the trial completed in 2019 can be found in Figure 6-2 in Appendix 2. Due to a sensor error during the 2018 trial environmental data are only presented for one trial.

To determine the population of *X. gardneri* on leaf surfaces, the foliage from all plants in each plot was harvested, and a leaf wash conducted using the protocol of Cuppels et al. (2006) with modifications. Due to variability between plants within and between plots, all plants in a plot were pooled for tissue sampling to ensure a minimum of eight grams of fresh material was obtained. From the harvested foliage two, four-gram samples were collected per plot, placed in tubes containing 40 mL of sterile distilled water and shaken for 30 minutes at 150 rpm. Samples were strained through Whatman GF/A 9 cm glass fibre filters (GE Healthcare, Chicago, Illinois, USA) before being split into four samples of 15 mL, each in sterile tubes, then centrifuged at 6000 rpm for 15 minutes. 13 mL of liquid was then removed from each sample before vortexing the samples and combining them back into a single sample of 8 mL. Centrifuging at 6000 rpm was repeated and 6 mL removed before the final sample was resuspended for dilution. Dilutions to 10^{-3} were made and each dilution plated on CKTM in duplicate (Sijam et al. 1991). In 2018 rifampicin amended TSA (RTSA) was also used as a plating medium along with CKTM, with each dilution plated on each medium in duplicate (Gevens et al. 2007). RTSA was dropped as a medium in 2019 due to contamination issues with other leaf microflora.

The number of X-like colonies was recorded three and five days after plating and the colony-forming units (CFU) per gram of fresh leaf tissue was determined. Colonies were identified by visual inspection by comparing the dilution plates to the isolate plated on RTSA and CKTM. Samples to confirm *X. gardneri* by DNA identification or to identify other colonies were sent to the PDC, as described in section 2.3.1.

2.3.3 Outdoor transplanter dissemination

One outdoor transplanter dissemination trial was completed in 2016, 2017 and 2018 at the University of Guelph Ridgetown Campus. To evaluate whether contaminated planting equipment influenced pathogen dissemination, tomato plants at predetermined locations, planted before or after the inoculation point, were assessed for the time period required for symptoms to appear. Tomato seedlings were produced as described in section 2.3.1. Seedlings with symptoms of bacterial spot were used as an inoculum source and produced as described in section 2.3.1.

The trial was arranged in a randomized complete block design with four replications per treatment; the dimensions of the field required the four blocks to be sequential, as shown in Figure 2-2. Each plot consisted of a twin row of tomatoes where one row consisted of all healthy plants and the second had treatment plants planted at the halfway mark. In 2016 the rows were only 45m long, but this was extended in 2017 and 2018 to 50m long so that assessed plants were not at the end of the row. Rows were spaced at the commercial standard 1.65m apart, with plants spaced 30 cm apart within rows. Each pair of twin rows constituted a plot, separated from neighbouring plots by a pair of corn rows, to provide a windbreak. Corn rows were planted May 10, May 11 and May 9 in 2016, 2017, and 2018 using cultivars 'MZ4010DBR', 'DKC49-82R1B', and 'NK0199-3122A-EZ1', respectively. Tomatoes were transplanted on May 30, June 2, and May 30 using a custom twin-row planter (RJ Equipment, Blenheim, ON). Corn rows were at 3-5 leaf stage when tomatoes were planted and approximately 40 cm high.

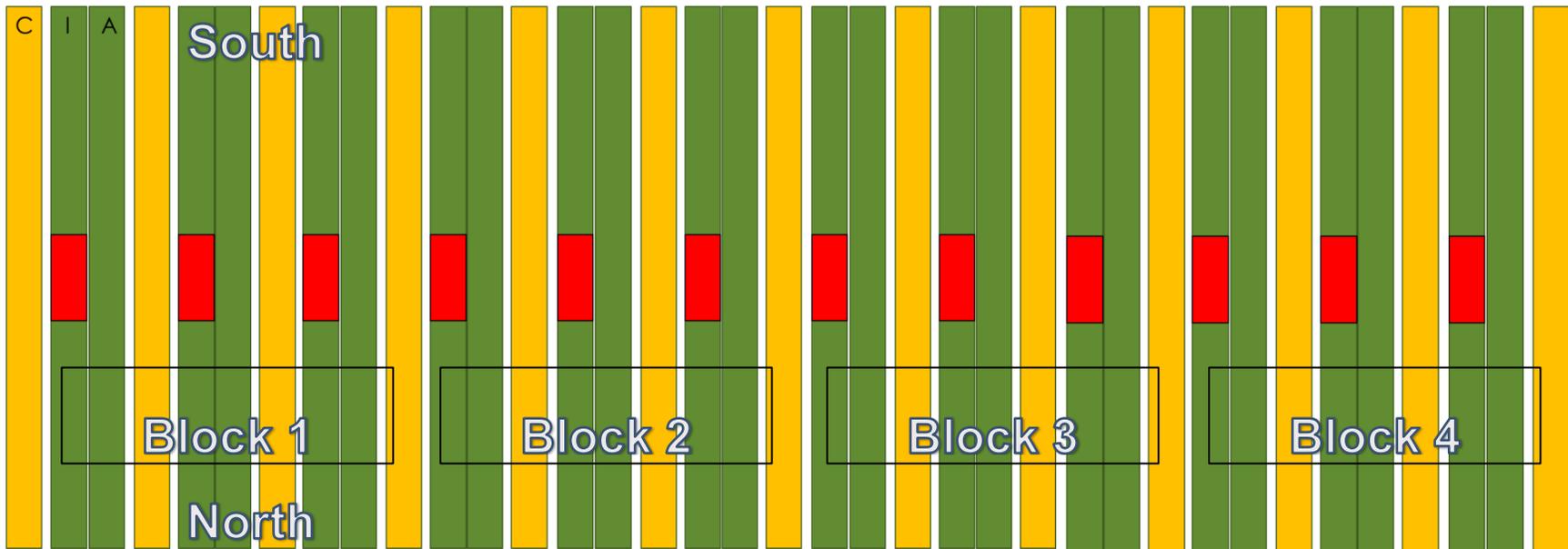


Figure 2-2 Design of transplanter dissemination trial

Corn rows (C) were used as plot isolation barriers, plots were randomized within each block and consisted of an inoculated (I) row and an adjacent row (A). Red boxes indicate the hypothetical placement of inoculated plants planted at the midpoint, with a minimum of fifty-five plants planted before and after the midpoint. When planting equipment moved from the south towards the north end.

Treatments included an all transplanter planted healthy control (C), a hand-planted disease control (H), and diseased transplanter treatment (T). To apply treatments, both twin-rows of the healthy control and HPC, and the adjacent row of the T were planted first. Following this, three plants North (N) and South (S) of the halfway point in the inoculated row were replaced with symptomatic plants and their position flagged (Figure 2-5). For the T the transplanter was stopped three plants before the halfway point in the inoculated row and the clean plants removed from the machine. Six diseased plants were placed in the carousels by a designated handler, then planted by the transplanter. Once the diseased plants passed through the machine their position in the row was flagged, and planting of healthy plants resumed. All T rows were planted in the same driving direction (driving south-to-north) so that the effect of driving direction was not a compounding factor and helped control any effect of wind during planting. In 2016 the T row in the third replicate was planted in the wrong twin-row with driving direction north-to-south. The transplanter was washed and disinfected between planting each T. The machine was rinsed then disinfected using a 20% Dettol (4.8% chloroxylenol) solution before it was rinsed again and dried with an air compressor. After planting in 2018, any extra tomato plants that remained were incubated in the vegetable lab under artificial lights. The plants were incubated for 14 days and then assessed for bacterial spot symptoms as described in section 2.3.1.

Plants located at the 5th, 10th, 25th, 40th and 55th plant from the inoculation point in both south (S) and north (N) directions of the inoculated row, the immediate north and south edge, 5th and 10th plants from the inoculation point in the adjacent row were

flagged and monitored every seven days for the appearance of bacterial spot symptoms. Symptomatic tissue was collected and bacteria isolated, as described in 2.2.1, to confirm the presence of *X. gardneri*. Samples were plated and colonies visually assessed until visual confirmation of X-like colonies, at which time samples appearing to be *X. gardneri* were sent to the PDC for confirmation by DNA analysis. Once *X. gardneri* was confirmed within a plot using these protocols sampling from that plot ceased, but assessments continued until all plants displayed bacterial spot symptoms. In 2016, only 95% of the assessed sampling points developed disease symptoms, plants located at positions N55, N40, N25, N10 in plot 101, N55, N40 in plot 102, N55 in plot 103, N55 in plot 303, N55, N40 in plot 401, and N55 in plot 403 did not develop bacterial spot symptoms that year. In both 2017 and 2018, disease symptoms were found in all assessed locations.

2.3.4 Statistical Analysis

All data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The analysis of variance of the least square (LS) means was performed using the GLIMMIX procedure, with a type I error rate set to 0.05. Tukey's Honestly Significant Difference, coupled with the ILINK statement, was used to separate the means and ensure values were always reported on the normal scale. The pdmix800 macro was used to present these differences (Piepho 2012). Shapiro-Wilk and AIC values were used to test the normality of residuals, and the distribution of errors was checked using residual plots. Covtest-WALD (Z test) was used to validate combining data for each set of trials. Unless stated otherwise, trial data were assessed on the normal scale.

Trailer dissemination trials were analyzed as a split-plot with the effects of the treatment, tray and the relationship between the two considered fixed effects. A mock trailer unit, containing an inoculum tray and four assessment trays of seedlings, constituted a plot and was treated with an irrigation treatment, while the four assessment trays, separated from the inoculum tray by set distances, created the split-plot. Since there were multiple repeats of the trial each year, the effects of each year and the nested relationship of each trial in a year were considered random. Additionally, the effect of the replicate and the effect of the replicate on the treatment were considered random. Data and standard error for the trailer dissemination trials were processed on the lognormal scale and returned to the normal scale using ILINK.

Indoor dissemination data from each year were run independently due to the decrease in colony-forming units between years, treatment was considered as the sole fixed effect. Because the study was done in a controlled environment under as consistent conditions as possible the year, replication, and replication-treatment relationship, were treated as random effects. Data and standard error for the indoor dissemination trials were processed on the normal scale.

Outdoor dissemination data from all years were pooled together, and data collected from each plant position analyzed individually by treatment. For each plant location the treatment was considered a fixed effect while the replicate, its relation to the treatment, and the year, were considered random effects. Data from plant positions S40, N5 and NC5 were run on the lognormal scale and back transformed using the ILINK statement based on the results of normality tests. Data and standard error for the

outdoor dissemination trials were processed on the normal scale, except for positions S40 and N5 in the inoculation row and N5 in the adjacent row which were processed on the lognormal scale and returned to the normal scale using ILINK.

2.4 Results

2.4.1 Trailer dissemination

Overall, symptom incidence ratings were low, and ranged from 0.1 to 0.8% on shelves placed at 91.5 (shelf C) and 30.5 (shelf A) cm below the inoculated tray, respectively. Based on symptom development, plants on shelf C had a disease incidence rate 87.5% lower than those on shelf A, while plants on shelf D had 75% fewer symptoms compared to shelf A. Shelf B had a symptom incidence statistically equivalent to shelves A and D, and symptom incidences on shelves C and D were also equivalent (Figure 2-3).

Both irrigated treatments had the same disease incidence rates overall, regardless of the direction of watering, and had higher disease incidence rates than the dipping method. Irrigating top to bottom or bottom to top resulted in 89.7 to 92.6% higher incidence rate respectively than did dipping the trays. The non-inoculated control was free of symptoms, and statistically lower than all three treatments (Figure 2-4). No symptoms were found on holdback plants.

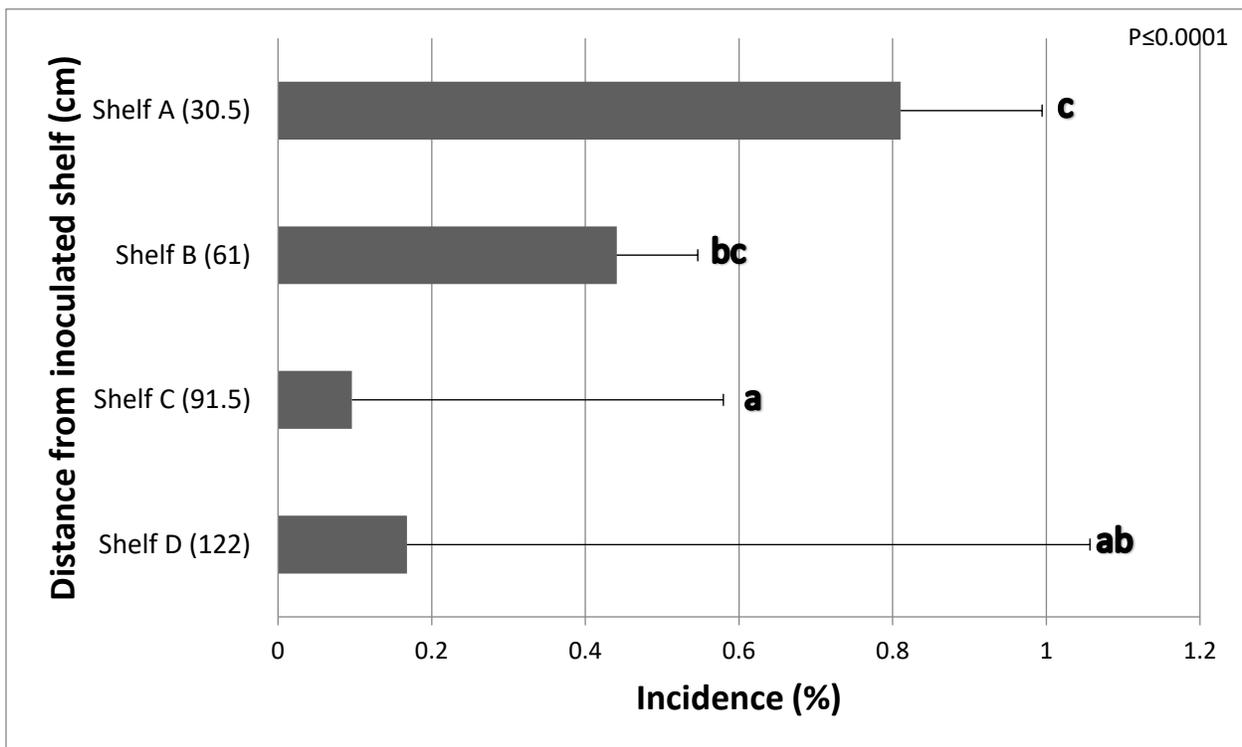


Figure 2-3 Effect of tray distance from inoculation source on the incidence of bacterial spot symptoms on processing tomato seedlings in a simulated irrigated tomato transport trailer. Results from four replicated trials at Ridgely Campus Greenhouse, 2017-2018, including data from control plots, with error bars for positive standard error of the mean. Data and standard error were processed on the lognormal scale and returned to the normal scale using ILINK. Bars followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD.

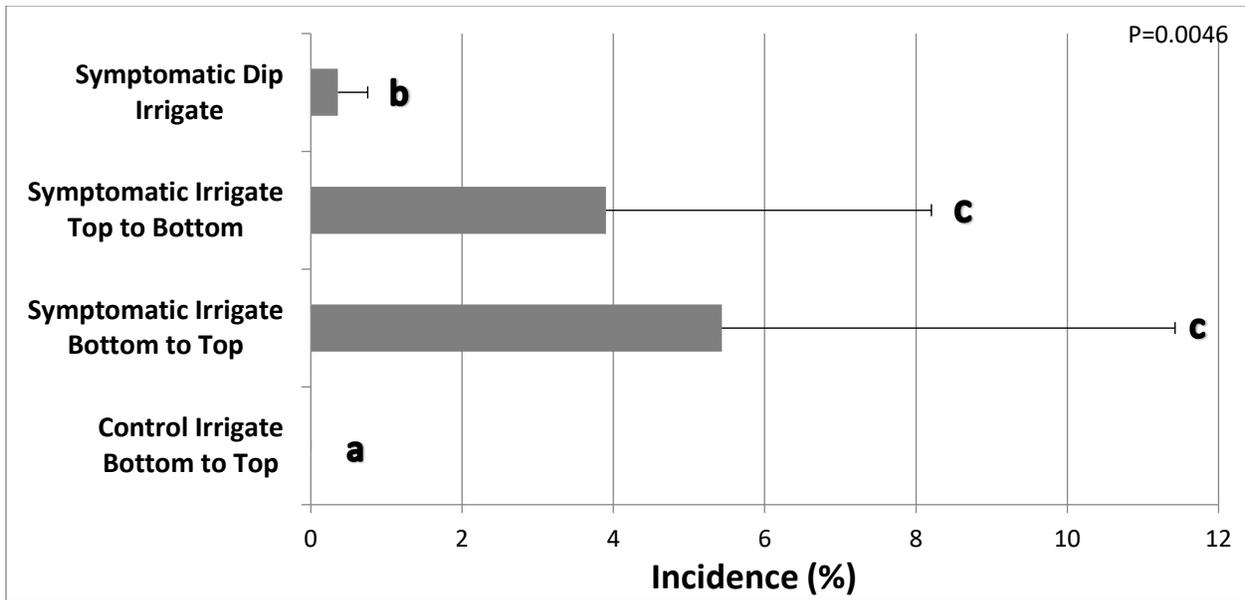


Figure 2-4 Effect of irrigation treatment on the incidence of bacterial spot on processing tomato seedlings in an irrigated tomato transport trailer.

Treatments included submerging plug trays, to the top of the tray, in a container of water for 60 seconds (Dip Irrigate) or irrigating with a hose for three to four seconds per tray in either the top to bottom or reverse direction. Results from four replicated trials, Ridgetown Campus Greenhouse, 2017-2018 with error bars for positive standard error of the mean are presented. Data and standard error were processed on the lognormal scale and returned to the normal scale using ILINK Bars followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD.

2.4.2 Indoor transplanter dissemination

No bacterial spot symptoms were observed in any plants exposed to *X. gardneri* via the transplanter. Plants used as the symptomatic inoculum and then incubated for the same 14 days remained symptomatic throughout and after incubation. No symptoms were found on holdback plants.

Colonies of *X. gardneri* were recovered from leaf washes of both pathogen exposed treatments (ie. dry and wet foliage), but not the healthy control. There was too much variability in the number of CFUs recovered to differentiate either treatment from the control in either trial (Table 2.1). In 2018 the number of CFU/g recovered from non-symptomatic treatment plants was 1,405 and 2,086 for dry and wet seedlings, respectively, passing through the planter compared to 158,793 CFU/g found on the symptomatic plants that had been put through the planter previously. The same trend was seen in 2019; only 1 and 5 CFU/g were recovered from dry and wet treatments respectively, but 14,489 CFU/g was recovered from the symptomatic plants. In both trials, samples grown on RTSA were identified as rifampicin resistant *X. gardneri* using the method described in section 2.3.1.

Table 2.1 Incidence of bacterial spot symptoms and *X. gardneri* population after five on semi-selective Chang Kama Tween Medium (CKTM) agar from processing tomato seedlings passed through transplanting equipment previously exposed to tomato seedlings with bacterial spot lesions, and inoculant plants. Results from two replicated trials, Ridgeway Campus, 2018-2019.

Treatment		Leaf Moisture	Incidence (%)	2018 Population (CFU/g fresh leaf tissue) ^a	2019 Population (CFU/g fresh leaf tissue) ^a
First six seedlings	Next 46 seedlings				
Symptomatic ^b	NA ^c	NA	100.0	158,793	14,489
Healthy	Healthy	Wet	0.00	0 a	0 a
Symptomatic	Healthy	Dry	0.00	1405 a	1 a
Symptomatic	Healthy	Wet	0.00	2086 a	5 a
SE ^d				708.16	1.37
P-value				0.0887	0.0730

^a Numbers followed by the same letter in a column are not significantly different at $P \leq 0.05$, as determined by Tukey's HSD, processed on the normal scale

^b Inoculum plants incubated as positive controls

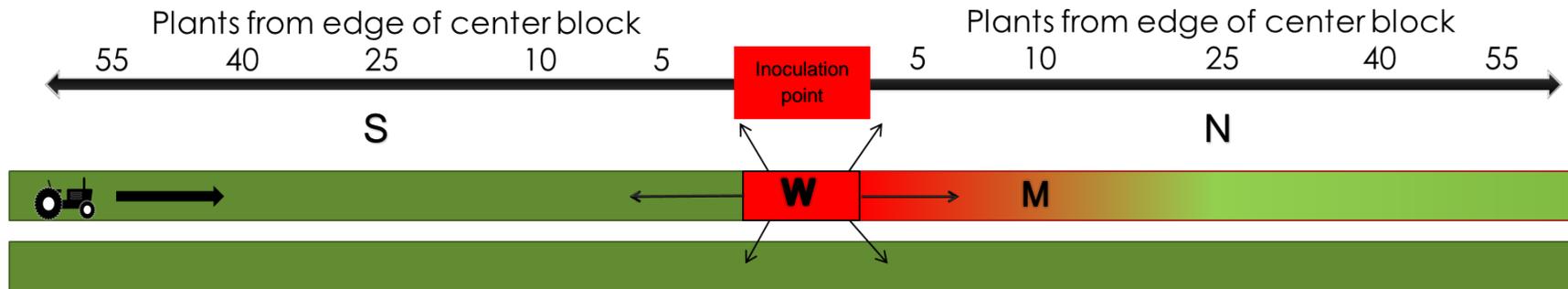
^c NA = Not Applicable

^d SE = Standard error of the mean

2.4.3 Outdoor transplanter dissemination

A range of 37 to 57 days to symptoms was observed in the field dissemination trial (Figure 2-5). Plants in the inoculated row of the T at position S5, before diseased plants were placed in the machine, developed symptoms earlier than either control. While at the N5 position, the T showed symptoms sooner than the clean control only, as it did at positions S10 and S25. At position N10 in the inoculated row of the hand inoculated treatment, symptoms developed sooner than the clean control and equivalent to the transplanted treatment.

In the adjacent row, all treatments were equivalent in their days to symptoms at the S10 and N10 positions. At the S5 position, both inoculation methods developed symptoms earlier than the clean control, while at the N5 position both treatments were equivalent with only the T having significantly fewer days to symptoms than the clean control. No symptoms were found on holdback plants incubated under artificial light in the Vegetable Research Facility.



Days to first symptoms – inoculation row

C	57 a	50 a	52 a	47 a	46 a		47 a	49 a	49 a	54 a	57 a
T	51 a	47 a	43 b	41 b	37 b		42 b	43 ab	49 a	52 a	56 a
H	51 a	47 a	48 ab	43 ab	43 a		39 b	43 b	47 a	50 a	56 a

Days to first symptoms – adjacent row

C				47 a	49 a		47 a	50 a
T				44 a	43 b		42 b	47 a
H				44 a	40 b		43 ab	47 a

Figure 2-5 Effect of transplanter movement on days to symptom development when plants with bacterial spot lesions pass through a tomato transplanter at plot center (inoculation point).

Predicted pattern of disease spread based on predicted influence of weather (W) or mechanical (M) driving direction, are shown in example plot. Tractor driving direction was South-to-North, numbers on the left represent the south side (S), before inoculation, while the right represents the north side (N), after the inoculation point. Rows were planted with either healthy control (C), machine transplanted diseased plants (T) or hand planted diseased plants (H). Results from three replicated trials, Ridgetown Campus, 2017-2018. Numbers in a column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD, analysis compared means among treatments at the same position. Data and standard error were processed on the lognormal scale, except for positions S40 and N5 in the inoculation row and N5 in the adjacent row which were processed on the lognormal scale and returned to the normal scale using ILINK. Red boxes designate the inoculation point in the inoculated row. Green boxes designate the same space in non-inoculated adjacent row East of the inoculation row, or control. Adapted from figure first conceptualized by J. LeBoeuf (former OMAFRA).

2.5 Discussion and conclusions

The major objective of this work was to assess the impact of early season activities on the spread of the tomato bacterial spot pathogen, *X. gardneri*. Transplants may be held in transplant trailers for eight to twelve hours if they are left overnight. In such cases, the environment is warm, humid and crowded with free moving water, ideal for bacterial movement (Goode and Sasser 1980). Transplanting equipment requires every transplant be picked up by a worker and passed through the cups in the carousel of the machine, giving two opportunities for bacteria to pass, via physical contact, from one host plant to another. No approved products currently exist to control tomato bacterial spot, so the reduction of the number of plants carrying the pathogen currently provides the only solution to mitigating potential epidemics later in the season (Trueman 2015a; Trueman and LeBoeuf 2015).

Both the distance from the inoculation point and the method of irrigation used on seedlings in a tomato trailer were assessed to discern their ability to spread the pathogen. When symptomatic seedlings were placed on the top shelf of a mock-trailer, disease symptoms could be found up to 122.0 cm from the inoculation point, on the lowest shelf, regardless of irrigation method. While seedlings 30.5 cm from the inoculation point, the first shelf below, had a significantly higher incidence rate than those 91.5 and 122.0 cm below, that does not reduce the impact of the trailer unit as a whole. Symptoms were concentrated near the inoculation point, but the epiphytic population of bacteria on non-inoculated plants was not enumerated, so the exact

extent of the contamination of the distant shelves is uncertain, but for practical sanitation the trailer must be treated as a whole contaminated unit.

Directly comparable work on the impact of water dissemination of pathogens in enclosed spaces was not found in reviewing the literature but works assessing the transmission of both *Xanthomonas perforans* and CMM in the greenhouse were consistent with those presented here. For CMM Hausbeck et al. (2000) found in the greenhouse, the pathogen was observed to spread across the entire 25 cm distance from one corner of the plot to the other when inoculated in opposite corners. They used overhead watering to maintain plants in that study; their pattern of spread is consistent with the pattern enabled by water movement found in the current trailer study (Hausbeck et al. 2000). Abrahamian (2017), showed that *X. perforans* spread across seedlings in trays an average of two and a half centimeters per day, reaching 179.1 cm away from the inoculation point in 28 days. With open surfaces and regular overhead watering a microbial pathogen was capable of significant movement across plants (Abrahamian 2017). Vallad (2019) recommended 1-2 trays would need to be removed from any symptomatic source to contain the spread due to its speed.

Water is a common vector of microscopic pathogens, but it is not limited to greenhouse environments (Miller et al. 2015). The dissemination pattern seen in the trailer irrigation trials also agrees with the field irrigation work done by Li *et al.* (2016), which showed that the flow of water is important for CGMMV transmission, with drip irrigation disseminating the pathogen significantly less than flooding in field irrigated watermelon (Li et al. 2016).

Ultimately, no method of irrigating the transplants stopped pathogen spread completely. Irrigation with running water from bottom to top versus from top to bottom made no difference in pathogen spread. Dipping plug trays in standing water for 60 seconds before placing them in the plug trailer did result in a 90 to 93% decrease in symptoms compared to irrigating from above or below the inoculation point, respectively. The increased time and labour costs of dip irrigating transplant trays, as well as sterilizing dip trays, should be assessed, as they may reduce the viability of this method, but to reduce the spread of pathogens in this environment dip irrigation should be the recommended method.

Detection of the disease consistently, and throughout the trailer unit, indicates that sanitation procedures should be developed and implemented by the industry to try to reduce residual bacteria remaining in the trailer between loads. These procedures, along with minimization of the time plants remain in the plug trailer, should also be treated as constitutive since the pathogen is not visible. This increases the necessity of clean seed in production to keep pathogen numbers low, and the need to sanitize equipment if the risk of spreading the pathogen at this stage will remain high.

Colonies of *X. gardneri* recovered from the indoor dissemination trial point to the transplanter as a viable transmitter for the bacteria, separate from workers hands. Statistical significance was not achieved due to the high variability between plots, and the too few data points that the leaf wash allowed. However, the consistent recovery of *X. gardneri* colonies from inoculated treatments is still concerning. The field dissemination trial however, showed that disease symptoms did not develop in the

pattern expected if the direction of the planting equipment was a significant factor and the controls were symptomatic within a few days of the rest of the trial, supporting the idea that rain and wind spread the pathogen throughout. While infection by bacterial spot originating from outside the trial cannot be completely ruled out, field and holdback plant monitoring do not support this idea. No symptoms developing on holdback plants indicates the seedlings used in the trial, which originated from the same lot, were free of the pathogen at the greenhouse stage, and in the field only one plant in three years was found to have been naturally infected with a different *Xanthomonad* than was used in the trial (*X. perforans*). Invasion of the trial by an externally originating *X. gardneri* strain would not be detectable, but the risk was reduced by minimizing the number of people working in the trial, ensuring that any work done was completed first thing in the morning, the trail was not re-entered on the same day and was planted each year as far from other *Solanum* trials as possible.

Taken together this points to the transplanter likely being a less concerning factor than the weather, as there was insufficient transmission by the machine to produce symptoms before weather transmitted bacteria did so. Sharabani *et al.* (2012) showed that when tools infested with CMM were used to trim greenhouse tomatoes the pathogen spread to the next four plants with just dry leaf surface contact, but that pathogen requires only one in 10,000 plants infected to facilitate an outbreak (Sharabani *et al.* 2013). Therefore, any implement making physical contact with plants should be considered for sanitation, an endeavour which would require research and marketing of effective sanitizers. This would likely result in the development and

adoption of more self sanitizing implements. Unfortunately, such endeavours remain unexplored at this time.

Holdback plants from all trials showed that no symptoms of disease originated from the seed or greenhouse environment after incubating for 14 days; it is unknown if the pathogen may have been present asymptotically. Currently seed is treated commonly using chlorine or hot water and certified using methods from the International Seed Federation (Lewis Ivey and Miller 2005; Dick and Dick 2014; International Seed Federation 2017). More work to control escapes is likely required at the end of the greenhouse stage where symptoms may or may not yet be identifiable before plants are moved to their final destination. Implementing leaf wash procedures on such a large scale would be both difficult and expensive, and only allow diagnosis of the problem, not prevention or remedy of it. There are sanitation procedures and products being developed and tested for pests of all types, and across many horticulturally significant plant species. Pathogens such as TMV, and CMM can be neutralized with products such as oxone sodium dodecylbenzenesulfonate sulfamic acid (Virkon®) and sodium hypochlorite, but could be prohibitively damaging to surfaces like trailer canvas or transplanter aluminium, thus requiring more research (Baysal-Gurel et al. 2015; Li et al. 2015; BCMA 2018). Automated sanitation, such as passing seedlings through a microwaving system on a conveyer before they leave the greenhouse facility, could also be a cost effective option in the future (Lozano et al. 1986). This would allow the automatic control of any escapes from the seed treatment stage that have since colonized the plants and would otherwise be disseminated. Microwaves as a sanitation

option have recently come back into consideration, and was shown to have some effectiveness against the fungus *Colletotrichum lindemuthianum* in dry beans (Friesen et al. 2014). Bacteria being a smaller pathogen, often with fewer effective chemical controls than fungal diseases, may prove more worthwhile for microwave technology development. With improved computer automation control, and the necessity to improve disease management, microwaving could potentially be incorporated as part of trailer loading procedures for greenhouse started plants (Paolanti et al. 2015).

Ultimately, pathogen favouring environmental conditions and the initial presence of the pathogen on the seed or plant are largely beyond the control of growers. Within their control is whether their equipment is sanitized between loads, at breaks, the end of the planting day or the end of the season. While further work is needed to assess products and procedures for optimal sanitization of these implements, the addition of the time and expense to do so should be looked at as a requirement to produce a healthy crop, as prevention remains the best solution (Goode and Sasser 1980).

3 Assessment of cultivar resistance to bacterial spot caused by *Xanthomonas gardneri* on foliage and fruit

3.1 Abstract

Bacterial spot of tomato (*Xanthomonas gardneri*) is increasingly becoming an issue for Ontario field tomato production, with resistance to copper-based pesticides prevalent. Even when chemical controls are available, host resistance remains important for integrated pest management, but tomato breeding has not produced a resistant phenotype and focuses on foliar health, which may not be sufficient. Anecdotal reports from the industry suggest that tomato fruit and foliar health are not mutually indicative of plant health in relation to bacterial spot. To investigate this difference, nine commercial cultivars were inoculated at the vegetative (foliar trial) or reproductive (fruit trial) stages from 2016-2018. In foliar trials, symptoms appeared at the same time in all cultivars in field and growth room trials. In field trials the standardized area under the disease progress curve (sAUDPC) for defoliation was 51 to 54% higher for 'TSH18' than 'H9706', 'Hypeel 696' and 'H3406', but equivalent to 'CC337', while fruit incidence was 49 and 47% lower for 'CC337' than 'TSH18' and 'H9706', but equivalent to 'H3406' and 'Hypeel 696'. Fruit severity was 63 and 60% lower for 'CC337' than 'H9706' and 'H3406', respectively, but equivalent to 'TSH18' and 'Hypeel 696'. In the fruit trial, fruit incidence was equivalent among cultivars in randomly sampled fruit, while the disease severity index for 'H9706' (3.4) was higher than 'Hypeel 696' (0.7). The observation that 'H9706' was less susceptible to defoliation than 'TSH18' but had equivalent fruit incidence and severity to 'TSH18' suggests differences in disease resistance between plant organs.

3.2 Introduction

Xanthomonas gardneri is the dominant causal agent of bacterial spot of tomatoes (*Solanum lycopersicum* L.) affecting growers in southwestern Ontario (Abbasi et al. 2015). Ontario field tomatoes had a farm gate value of \$52 million in 2017 from over 470,000 harvested tons of tomatoes, but a conducive season for bacterial spot can result in up to 60% of that yield lost (LeBoeuf et al. 2009; OPVG 2018). Yield reductions occur when the foliar lesions of bacterial spot cause defoliation, reducing productivity, while lesions that develop on the fruit reduce their quality and value (LeBoeuf et al. 2009). From the 1980s to the mid-2010s copper-based sprays were the standard management practice for controlling bacterial spot but sprays needed to be applied often, allowing the pathogen to developed resistance (Zevenhuizen et al. 1979; Conover and Gerhold 1982). Copper-based sprays are no longer a recommended management strategy in Ontario (OMAFRA) (Trueman 2015a; Trueman and LeBoeuf 2015).

There are four species of the *Xanthomonas* genus which cause tomato bacterial spot in Ontario; *X. euvesicatoria* (Jones, Lacy, Bouzar, Stall & Schaad), *X. vesicatoria* ((Doidge) Vauterin, Hoste, Kersters & Swings), *X. perforans* (Jones, Lacy, Bouzar, Stall & Schaad), and *X. gardneri*, which represented 1, 2, 26 and 36% of the Ontario population, respectively, in a recent survey (Abbasi et al. 2015). Multiple species in a geographic region allow the population to shift when selective pressure mounts, which occurred when *X. gardneri* supplanted *X. perforans* as the dominant species in Ontario (Cuppels et al. 2006; Abbasi et al. 2015). Within the state of Indiana, different species

can be dominant in relatively close proximity, potentially requiring multiple species-specific management strategies in a single area (Egel et al. 2018).

These pathogens have limited ability to overwinter in Ontario and surrounding areas but arrive annually on transplants grown from contaminated seed (Bashan et al. 1982; Cuppels et al. 2008; Ma 2015). This makes managing the annual reintroduction of the pathogen difficult and supports the concept of host resistance as a method to improve management, though the development of host resistance to this disease has been difficult.

Traditionally, host resistance is an important part of plant disease management and is usually achieved using qualitative resistance (Vale et al. 2001). These genes can be tagged and mapped using genetic technology, making them relatively easy to work with and follow in breeding lines (Foolad 2007; Hutton 2008; Wang et al. 2011). Unfortunately, major resistance genes do not exist for BSX. In such cases host resistance relies on quantitative resistance genes, manifesting in a gradient of resistance rather than all or none.

Quantitative resistance is more difficult to develop because of the number of genes required, but is also more durable over time than qualitative resistance (Vale et al. 2001; Foolad 2007; Hutton 2008; Wang et al. 2011; Scott et al. 2015). Some quantitative resistance genes or alleles are also associated with traits like small fruit and may need to be replaced with other genes. This can complicate the process of breeding

for host resistance against BSX, as producers will not make use of a cultivar whose fruits are too small or lack qualities that consumers expect (Scott et al. 2015).

Another potential complication is the host itself, with anecdotal reports from industry suggesting that the fruit of some processing tomato cultivars is more susceptible to BSX than its foliage when compared to other cultivars, or that fruit lesions on some cultivars are more severe, regardless of foliar incidence (C. Trueman, pers. comm.). Xanthomonad infection of foliage is well documented, with effectors identified that are believed to force open stomata that have been closed by the hosts' innate defenses (Melotto et al. 2008; Schornack et al. 2008; Gudesblat et al. 2009b). Infection of fruit however, remains under debate, with fruit trichomes and flowers believed to act as entry points (Getz 1983; Bashan and Okon 1985).

Foliar evaluations do not require the laborious harvesting or sorting of fruit evaluations, but could be selecting for only foliar health if it is not directly linked to the health of the fruit. Differences between foliar and fruit incidence can sometimes be seen in product efficacy reporting, but it has been rarely reported on in detail (Roer and Toxopeus 1961; Peters et al. 1999; P. A. Abbasi et al. 2002; Abbasi and Weselowski 2015). In the 1960s disassociations between foliar and tuber health in potatoes (*Solanum tuberosum* L.) were reported in cultivars that carried major resistance genes against *P. infestans* (Roer and Toxopeus 1961). Their recommendation was tubers at various growth stages be directly assessed after inoculation with the pathogen as part of cultivar selections, instead of focusing strictly on foliar health. Later that would be expanded as differences between the flesh penetration by *P. infestans*, and skin health

of tubers was reported, showing that resistance may not be consistent across tissue types (Peters et al. 1999). Similar effects have not been investigated for BSX in tomato.

Since the link between fruit and foliage health in tomatoes remained untested and sparsely documented, the objective of this chapter was to establish if foliar disease measurements, specifically those derived from defoliation, had any relation to symptom development on fruit by evaluating both these fruit and foliar variables. A dissociation between foliar and fruit resistance within cultivars was hypothesized, namely that fruit symptoms would show more variability than foliar symptoms between processing cultivars. Based on field results, three cultivars showing the most deviation between bacterial spot symptom development on foliage and fruit were evaluated under controlled conditions.

3.3 Materials and methods

Each year two parallel field trials were conducted. One trial had the foliage inoculated with *X. gardneri*, while the other had the fruit inoculated, to investigate potential differences in foliar and fruit resistance within commonly grown commercial processing tomato cultivars. These were repeated over three years (2016 to 2018), using cultivars ‘CC337’ and ‘N3306’ (Conagra Brands, Dresden ON), ‘H5108’, ‘H9706’, ‘H1178’, and ‘H3406’ (HeinzSeed, Leamington, ON), ‘Hypeel 696’ (Seminis Vegetable Seeds Inc. Santiago Chile), and ‘TSH28’ and ‘TSH18’ (Tomato Solutions, Chatham, ON). Cultivars were seeded in 288-cell trays and grown in a local commercial greenhouse using standard production practices. Trays of started plants were removed from the greenhouse and brought to the University of Guelph, Ridgetown Campus and

stored outdoors before planting. Trials were transplanted on May 24, 2016, May 24, 2017 and May 24, 2018 using a custom transplanter (RJ Equipment, Blenheim, ON). Each plot consisted of a seven-meter twin row planter on 2-m centers, with an in-row spacing of three plants per meter. Each plot was separated by one guard row, consisting of 'Hypeel 696' plants.

Only one validation trial of the fruit results was completed in 2019 due to the time required for fruit production in a controlled environment, while two validation trials of foliar results were completed. All validation trials used a reduced cultivar list, 'CC337', 'H9706' and 'TSH18'. Field trials were completed at the University of Guelph, Ridgetown Campus, Ridgetown Ontario (42°26'55.9" N 81°53'05.3" W) and controlled environment trials were completed in the Ridgetown Campus greenhouse and growth room. All trials were set up in a randomized complete block design with four replications. Minimum and maximum daily temperature and rainfall data for field trials were obtained from the weather station located at Ridgetown Campus through Environment Canada. Validation trials were monitored hourly for minimum and maximum temperatures and relative humidity, using a HOBO MX2305 Temperature Data Logger (Onset Computer Corporation, Bourne MA). Environmental data for all trials is presented in Appendix 2.

3.3.1 Foliar inoculation and assessments

3.3.1.1 Field trial

Foliar trials were inoculated with a solution of *X. gardneri* DC00T7A (D. Cuppels, London ON) at a concentration of 1×10^6 CFU/mL using ULD 120-02 nozzles and a water volume of 200 L/ha, seven to ten days after planting. Inoculation occurred ten days after planting in 2016 and eight days after planting in 2017. In 2018 inoculation initially occurred eight days after transplanting but plots were re-inoculated twenty-two days after transplanting due to dry conditions, no symptoms developed from the initial inoculation in 2018.

Plots were visually assessed for bacterial spot symptoms daily beginning seven days after inoculation. Five plants from each plot were randomly selected, and every leaflet visually inspected each day until symptoms were observed in the plot. To confirm diagnosis suspected symptoms were collected, sampled and plated on TSA (Fisher Scientific, Canada) and CKTM, which is semi-selective for *Xanthomonas*, to confirm that colonies phenotypically resembled *X. gardneri*, as outlined in section 2.3.1, (Sijam et al. 1991; Cuppels et al. 2006). Each year, representative samples were sent to the Pest Diagnostic Clinic (PDC) at the University of Guelph, Laboratory Services (Guelph ON) to confirm colony identification using Amplified Fragment Length Polymorphism primers originally developed by Koenraadt et al. (2009), to differentiate BSX species (Koenraadt et al. 2009). Isolates whose identity was uncertain were also sent for identification. Once symptoms were confirmed, daily assessment of affected plots ceased.

Plots were also monitored for defoliation weekly using Kousik's scale modified with five percent increments (Kousik et al. 1996). Due to the large assortment of early, mid and late-season cultivars, defoliation values within different DBH ranges were used to calculate the AUDPC. Defoliation values 43 to 47 DBH, 32 to 36 DBH, 20 to 26 DBH, and 11 to 17 DBH were used to calculate the AUDPC using the following equation where Y_i is percent defoliation at day X_i , and Y_{i-1} is percent defoliation at day X_{i-1} (Plank 1963);

$$\text{AUDPC} = \sum [(Y_i + Y_{i-1}) (X_i - X_{i-1})]/2 \quad (1)$$

Which was standardized for the length of assessment period with (Duveiller et al. 2005):

$$\text{sAUDPC} = \text{AUDPC}/\text{number of days} \quad (2)$$

Individual plots were harvested by hand when fruit reached approximately 90% red. A two-meter section of the plot was randomly selected for harvest. Red fruit, green fruit, and rots were separated and weighed before a random 100-fruit subsample of red fruits was collected. Fruits were assessed for incidence of bacterial spot by separating them into the following categories: 0 = no bacterial spot, 1 = 1 spot, 2 = 2 spots, 3 = 3 spots, 4 = 4 spots, 5 = 5 or more spots. A disease severity index (DSI) was calculated using the following equation (Kobriger and Hagedorn 1983);

$$DSI = \frac{\sum [(class\ no.) (no.\ of\ fruit\ in\ each\ class)]}{(total\ no.\ fruit\ per\ sample) (no.\ classes - 1)} \times 100 \quad (3)$$

The fruit was then re-sorted into additional categories which included “Small” lesions <5 mm diameter, “Split” lesions with skin broken open within the spot, “Large” lesions >5mm diameter, and “Not split” lesions where the skin was intact. Incidence was calculated for each severity category.

3.3.1.2 Growth room trial

A growth room trial to validate results from the field trial was set up in a growth room at the University of Guelph, Ridgetown Campus and repeated twice. Three cultivars were seeded in 25-cell trays and grown in the greenhouse of the University of Guelph, Ridgetown Campus. Due to low natural light conditions during the fall of 2018 artificial lights were used. Ceramalux C400S51 bulbs (PHILIPS, Amsterdam, Netherlands) were installed on fixtures and timers built into the facility, running from 6 am to 10 pm. Once the plants were six weeks old, they were moved from the greenhouse to the growth room.

The trial was set up on two large metal shelving units each with three shelves. The first unit measured 180.34 cm long by 54.61 cm wide, with each shelf having 38.1 cm of clearance to the lights. The unit was constructed from a metal frame and custom-built wooden shelves which had a 6.35 cm deep lip and were lined with plastic, the second unit had shelves consisting of metal trays (177.8 cm x 60.96 cm) with a 5.08 cm deep lip and 45.72 cm of clearance to the lights. A pair of T5 LED lights were

suspended above each shelf and ran on timers to provide a 16-hour photoperiod (6 am – 10 pm). The temperature of the growth room was set to 24°C and monitored by a HOBO MX2305 Temperature Data Logger (Onset Computer Corporation, Bourne MA). Plastic dividers were used to separate the plots so that each replication could fit on a single shelf without cross-contamination of plots.

Plants for each trial were seeded directly into 25-cell plastic tomato trays and grown for eight weeks. A 25-cell control tray, consisting of five plants of each cultivar, was also set up for each trial to act as holdbacks and be assessed for any latent disease. Only one control tray was established due to space restrictions in the growth room. Once established the plants were moved to the growth chamber, placed on metal shelves, and watered by flooding the shelves overnight to ensure they did not dry out during inoculation while leaves remained dry to minimize any movement of the pathogen between the plants.

Trays were inoculated by spraying both the upper and lower leaf surface until run-off, using a solution of 0.01% Sylgard, 1×10^7 CFU/mL *X. gardneri* DC00T7A (D. Cuppels, London ON) and distilled water in a small hand pump sprayer (Home Hardware Stores Ltd, St. Jacobs ON, 5050190). The inoculated trays were then covered in transparent plastic bins (Rubbermaid L0-2223-C2-WHT) for 24 hours. The control tray in each trial was sprayed with a solution of distilled water and 0.01% Sylgard. To prevent cross-contamination with inoculated trays on the day of inoculation, the control tray was sprayed with sterile distilled water and covered by a container but was left by a vent window outside the room. This was done to maintain access to light

while avoiding contaminated aerosols due to space restrictions in the room. After the 24-hour inoculation period the plastic bins were removed, and the trays, beginning with the control tray, were replaced on their respective shelves.

After inoculation, all trays were visually inspected daily for bacterial spot symptoms (see Figure 5-1 in Appendix 1 for reference image). To maintain humidity in the growth room, distilled water was sprayed on the plants using a bottle sprayer every other day. Once observed the first lesions were photographed, sampled, and plated on TSA as described in section 2.3.1 to confirm colony morphology. The first colonies consistent with *Xanthomonas* sp. were then sent for confirmation by PCR as described in section 2.3.1. Once a plot was confirmed symptomatic it was no longer inspected. Inspections of the trial continued until all plots showed symptoms consistent with the reference photographs. Once all replicates were symptomatic, the number of days to symptoms (DTS) was calculated.

3.3.2 Fruit inoculation and assessments

3.3.2.1 Field trial

On the day of inoculation, two reproductive clusters with green fruit and two with open flowers but no set fruit in each plot were marked with a number using orange ribbon. Records were kept for each numbered cluster which included the number of fruits, and the number of flowers that were spent, aborted, open or closed. In the evening the marked clusters were inoculated in each plot with a solution of *X. gardneri* DC00T7A (1×10^6 CFU/mL) and distilled water using a small hand pump sprayer (Carter's Home Hardware, Ridgeway ON, 5050190). Inoculation occurred twice, once

in early July and again in mid-July (July 7 and 19 2016, July 4 and 11 2017, July 4 and 11 2018) so that a total of eight reproductive clusters were marked and sprayed per plot. In addition, during each inoculation event, ten control clusters of fruit and flowers were marked and inoculated with distilled water in every other plot to act as controls. Controls were scattered throughout the trial to maximize their isolation from inoculated clusters. Only ten controls per inoculation were made to ensure enough clusters were available for both inoculation timings.

Beginning in mid-August plots were assessed weekly for fruit ripeness. Marked clusters were harvested and bagged when the cluster reached uniform ripeness, and 100 fruit plot samples were harvested when the plot reached 90% ripe. From each tagged cluster, the total number of fruit and number of fruits with bacterial spot symptoms were categorized and recorded, and the DSI calculated as described in section 3.3.1.1.

To compare the effect of the point inoculation to general fruit infection, the 100 fruit plot samples were similarly evaluated using a DSI calculation. The plot sample was taken randomly areas described in 3.3.1.1. If there were not enough fruit from the first pick, a new section of fruit was harvested by repeating the harvesting protocol.

3.3.2.2 Greenhouse trial

To evaluate fruit resistance indoors, the three selected cultivars were grown in 200-cell trays in the greenhouse of the University of Guelph Ridgetown Campus and grown as described in section 3.3.1.2. At six weeks old, two seedlings per plot were

then transplanted into 15-L buckets (Home Hardware, St. Jacobs ON, 4544001) filled with Miracle-Gro All Purpose Potting Mix (Scotts Miracle-Gro, Marysville Ohio, U.S.A.). Five replicates of each plot were planted, and plants were maintained until fruit set. Plants were secured using bamboo stakes and plastic ties to keep them upright. Due to a wiring error in the greenhouse, the grow lights (Ceramalux C400S51 bulbs (PHILIPS, Amsterdam, Netherlands)) were left on constantly for the first six weeks of trial establishment.

Once fruit set occurred in all plots, four reproductive clusters per plot were marked with a number using orange ribbon. From each numbered cluster, records were kept in the same fashion as described in 3.3.2.1.

After inoculation, marked clusters were monitored daily, each truss was harvested when all fruit in the cluster reached uniform ripeness. From each marked cluster, the total number of fruit and number of fruits with bacterial spot were categorized and recorded as described in section 3.3.1.1.

3.3.3 Statistical analysis

The data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The analysis of variance was performed with PROC GLIMMIX ($P \leq 0.05$). Tukey's Honestly Significant Difference ($P \leq 0.05$) was used to separate the means for every trial. Location, year, and replicate were treated as random effects. Shapiro-Wilk and AIC values were used to test the normality of residuals, and the distribution of errors was

assessed using residual plots. Covtest-WALD (Z test) was used to validate combining data for the field and growth room trials, respectively.

For the field foliar resistance data, only the days to symptoms and spot incidence data for “Any” spots on fruit in the 100-fruit random sample, the percent of fruit with large lesions and percent of fruit with not split lesions were run on the normal scale. All other data categories from the field foliar trials were processed on the log scale and back transformed using the ILINK statement. Data from the foliar validation trials were run on the normal scale.

Of the variables collected in the fruit inoculated trails, abortion data were run on the normal scale (Appendix 3), all other data were run on the log scale and back transformed using the ILINK statement, this included cultivar H1178, which was short one plot in the 2018 field fruit trial due to a shortage of transplants. Data from the greenhouse fruit validation trial were not processed.

Ranked correlation tests were done using Spearman’s Rho for foliar (AUDPC and sAUDPC) and fruit lesion variable pairings, using PROC COR. Foliar variables from the foliage inoculation trials were compared to lesion variables from both the foliage and fruit trials to account for differences in inoculation techniques between the trials (Appendix 3). 100 fruit random sample lesion data were used for all fruit lesion comparisons, either from foliar or fruit trials. Data was sorted by treatment order in either the foliar or fruit trial. None of the relationships were consistently strong ($r \geq 0.8$) and statistically significant at $P \leq 0.05$ across cultivars, and when comparing between

foliar data and fruit trial variables the relationships were affected by which randomization was used to sort the data. Because of this significant variability only strong relationships, defined as $r \geq 0.8$ for strong positive relationships or $r \leq -0.8$ for negative relationships, that demonstrated significance at $P \leq 0.05$ were considered.

3.4 Results

3.4.1 Cultivar foliar resistance

3.4.1.1 Field results

There were no differences ($P > 0.05$) among cultivars in the number of days to symptoms in the foliage (Figure 3-1). When percent defoliation was assessed based on DBH, it differed between three cultivars at only two points in time (Table 3.1). All cultivars showed the same percent defoliation early in the season, but at the last two time frames analysed, 20 to 37 DBH and 4 to 17 DBH, differences were observed between 'TSH18', 'H1178' and 'Hypeel 696', and then 'TSH18', 'H9706' and 'Hypeel 696', respectively (Table 3.1). At 20 to 37 days before harvest the percent foliar disease of 'TSH18' and 'H1178' was nearly three times higher than that of 'Hypeel 696'. At 4 to 17 DBH the percent foliar disease for 'H9706' and 'TSH18' dropped to 49 and 57%, respectively, higher than for 'Hypeel 696'. A smaller series of differences could be found in overall sAUDPC values that were established at the end of the season, where the sAUDPC values of 'H9706', 'Hypeel 696' and 'H3406' were approximately half that of 'TSH18', but all other cultivars had similar sAUDPC values, rendering them indistinguishable from the low or high sAUDPC groups (Table 3.2).

For the fruit symptom measurements at the harvest of the foliar trial, DSI of cultivars 'H3406', and 'H9706' were 6 and 7 points higher than 'CC337', respectively ($n=100$, Table 3.3). Additionally, 'N3306' had a DSI 56% lower than that of 'H9706'. Cultivar 'CC337' also had fewer than half as many lesions of "Any" size or type than either 'H9706' or 'TSH18'. There was no variation between cultivars in the incidence of

fruit with “Small” lesions (<5 mm) or those with “Split” lesions. In contrast, the incidence of “Large” lesions (>5 mm) for ‘CC337’ cultivar was 56, 60 and 62% lower than for ‘H1178’, ‘H9706’, and ‘TSH18’, respectively. Incidence of fruit lesions that were “Not split” were 65 to 76% greater in ‘H9706 and ‘H3406’ cultivars than in ‘CC337’ and ‘N3306’ (Table 3.3). When lesions were reanalysed as a percentage of diseased fruit, rather than total fruit harvested, there were no differences among cultivars (Table 3.4).

No symptoms were found on holdback plants after two-week incubation in the Vegetable Research Facility.

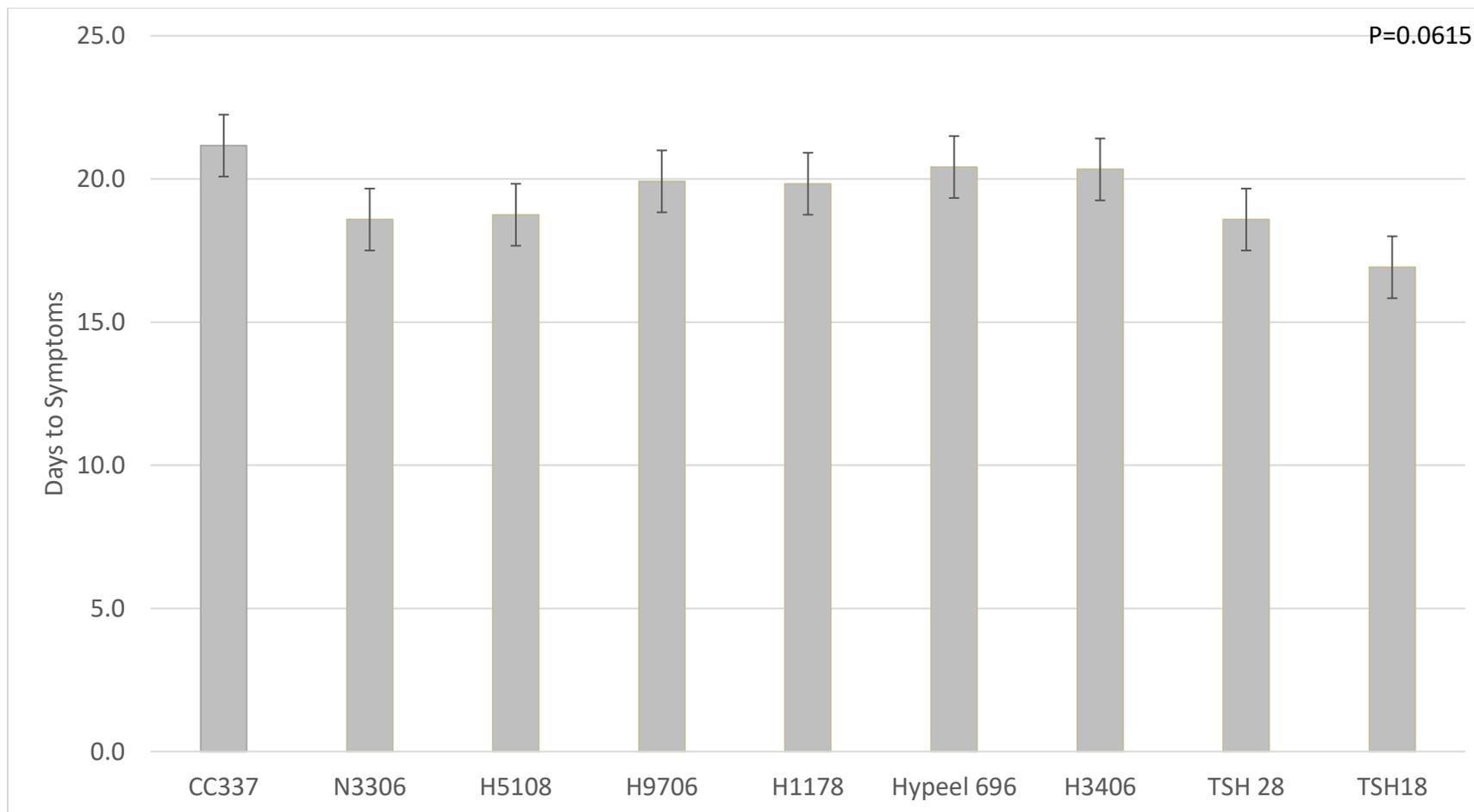


Figure 3-1 Days to first bacterial spot symptoms (DTS) on leaves of nine commercial processing tomato cultivars inoculated with *X. gardneri* seven to ten days after transplanting.

Foliar resistance trials completed at the University of Guelph, Ridgetown Campus, Ridgetown, ON, 2016 – 2018. Error bars represent standard error of the mean. Bars without letters are not significantly different at $P \leq 0.05$, Tukey's HSD. Data and standard error were processed on the normal scale.

Table 3.1 Progression of defoliation of nine cultivars caused by foliar bacterial spot disease, standardized by DBH. Data obtained from nine tomato cultivars foliar inoculated with *X. gardneri* seven to ten days after transplanting in foliar resistance field trials completed at the University of Guelph, Ridgetown ON, 2016-2018.

Cultivar	Defoliation standardized by DBH ^{a, b, c}							
	35 to 52	SE ^d	24 to 44	SE	20 to 37	SE	4 to 17	SE
CC337	2 a	0.8	8 a	2.3	25 ab	5.8	61 ab	8.6
N3306	4 a	1.3	12 a	3.4	29 ab	6.7	64 ab	9.0
H5108	3 a	0.9	7 a	1.9	31 ab	7.3	65 ab	9.2
H9706	4 a	1.0	11 a	2.8	40 ab	9.2	73 a	10.3
H1178	3 a	0.9	9 a	2.3	42 a	9.6	70 ab	10.0
Hypeel 696	2 a	0.7	6 a	1.8	16 b	3.8	49 b	6.9
H3406	6 a	2.0	11 a	3.2	35 ab	8.0	69 ab	9.8
TSH28	1 a	0.5	9 a	2.3	31 ab	7.2	66 ab	9.3
TSH18	2 a	0.9	15 a	3.9	47 a	10.8	77 a	10.9
P-Value	0.0900		0.3566		0.0220		0.0266	

^a DBH = Days before harvest.

^b Data analyzed using the lognormal distribution to meet assumptions of normality.

^c Numbers in a column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD data and standard error were processed on the lognormal scale and returned to the normal scale using ILINK

^d SE = standard error of the mean.

Table 3.2 Standardized area under the disease progress curve (sAUDPC) for defoliation of nine commercial processing tomato cultivars inoculated with *X. gardneri* seven to ten days after transplanting. Foliar resistance trials completed at the University of Guelph, Ridgetown Campus, Ridgetown, ON, 2016 – 2018.

Cultivar	sAUDPC ^{a, b, c}	SE ^d
CC337	10.7 ab	5.59
N3306	14.8 ab	7.48
H5108	13.5 ab	6.80
H9706	10.4 b	5.22
H1178	10.8 ab	5.43
Hypeel 696	9.6 b	4.83
H3406	10.1 b	5.10
TSH28	14.9 ab	7.49
TSH18	20.9 a	10.56
P-Value	0.0079	

^a Data analyzed using the lognormal distribution to meet assumptions of normality.

^b Numbers in a column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD, data and standard error were processed on the lognormal scale and returned to the normal scale using ILINK.

^c sAUDPC is calculated from defoliation values for each cultivar using their respective DBH defoliation values where Y_i is percent defoliation at day X_i , and Y_{i-1} is percent defoliation at day X_{i-1}

$$\text{AUDPC} = \sum [((Y_i + Y_{i-1}) (X_i - X_{i-1}))/2] \text{ standardized for the length of assessment period becomes:}$$

$$\text{sAUDPC} = \text{AUDPC}/\text{number of days}$$

^d SE = standard error of the mean.

Table 3.3 Incidence of Any, small (<5 mm), large (≥5 mm), “Split”, and “Not split” bacterial spot lesions on fruit, and associated disease severity index (DSI) for bacterial spot, on 100 red fruit randomly subsampled from a 2 m section of twin-row in nine tomato cultivars foliar inoculated with *X. gardneri* seven to ten days after transplanting. Data collected from foliar resistance field trials completed at the University of Guelph, Ridgetown ON, 2016-2018.

Cultivar	DSI ^{cd}	SE ^e	Incidence (%) ^{a,b}									
			Any	SE	<5 mm	SE	≥ 5 mm	SE	Not Split	SE	Split	SE
CC337	4.0 c	1.08	15.6 b	8.47	4.5	1.08	6.3 b	4.67	5.0 b	1.71	7.1	5.00
N3306	4.8 bc	1.08	20.7 ab	8.47	4.5	1.08	8.9 ab	6.67	3.9 b	1.32	8.1	5.71
H5108	5.5 abc	1.08	22.6 ab	8.47	4.4	1.00	10.8 ab	8.04	6.9 ab	2.34	7.7	5.44
H9706	10.8 a	1.08	29.6 a	8.47	7.1	1.54	15.9 a	11.78	13.4 a	4.59	8.6	6.09
H1178	7.6 abc	1.08	23.3 ab	8.47	3.8	0.83	14.4 a	10.72	8.0 ab	2.74	8.0	5.65
Hypeel 696	4.9 abc	1.08	18.3 ab	8.47	3.4	0.75	7.9 ab	5.88	6.3 ab	2.14	6.2	4.36
H3406	10.1 ab	1.08	26.6 ab	8.47	6.2	1.36	12.4 ab	9.22	12.8 a	4.36	7.6	5.42
TSH28	7.3 abc	1.08	27.5 ab	8.47	5.8	1.33	10.8 ab	8.05	7.5 ab	2.59	11.9	8.44
TSH18	7.5 abc	1.08	30.5 a	8.47	6.5	1.66	16.7 a	12.48	7.4 ab	2.56	15.8	11.32
P-Value	0.0013		0.0019		0.2580		0.0003		0.0003		0.0571	

^a The percent of a 100 fruit random sample with any bacterial spots, spots larger or smaller than 5 mm, “Split” or “Not split”.

^b Numbers in a column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey’s HSD, data and standard error were processed on the lognormal scale and returned to the normal scale using ILINK.

^c DSI is the relative weight of the number of lesions, calculated as;

$$DSI = \frac{\sum [(class\ no.) (no.\ of\ fruit\ in\ each\ class)]}{(total\ no.\ fruit\ per\ sample) (no.\ classes - 1)} \times 100$$

^d Data in this column were analyzed using the normal distribution.

^e SE = standard error of the mean.

Table 3.4 Incidence small (<5 mm), large (≥5 mm), “Split”, and “Not split” bacterial spot lesions, calculated as a percent of symptomatic fruit only. Calculated from 100 red fruit subsamples randomly taken from a 2 m section of twin-row in nine tomato cultivars foliar inoculated with *X. gardneri* seven to ten days after transplanting. Data collected from foliar resistance field trials completed at the University of Guelph, Ridgetown ON, 2016-2018.

Cultivar	Incidence (%) ^{a, b}							
	<5 mm ^c	SE ^d	≥5 mm	SE	Not Split	SE	Split ^c	SE
CC337	33.9 a	0.45	61.7 a	14.82	44.5 a	13.70	55.1 a	11.87
N3306	33.0 a	0.45	62.5 a	14.82	57.9 a	13.70	62.3 a	13.44
H5108	26.6 a	0.45	71.9 a	14.82	63.1 a	13.70	49.2 a	10.62
H9706	28.2 a	0.45	64.9 a	14.82	54.6 a	13.70	37.7 a	8.14
H1178	18.9 a	0.45	74.0 a	14.82	53.6 a	13.70	42.5 a	9.31
Hypeel 696	24.2 a	0.45	62.4 a	14.82	59.0 a	13.70	43.5 a	9.38
H3406	25.6 a	0.45	64.0 a	14.82	61.5 a	13.70	35.3 a	7.86
TSH28	30.2 a	0.45	60.3 a	14.82	52.5 a	13.70	63.2 a	13.84
TSH18	25.6 a	0.46	64.8 a	14.82	60.8 a	13.70	63.8 a	14.89
P-Value	0.4772		0.8338		0.6969		0.0047	

^a The percent of symptomatic fruit from a 100-fruit random sample with any bacterial spots, spots larger or smaller than 5 mm, “Split” or “Not split”.

^b Numbers in a column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey’s HSD.

^c Data in this column were analyzed using the lognormal distribution to meet assumptions of normality and back transformed using ILINK.

^d Standard error of the mean

3.4.1.2 Growth room foliar results

There were no differences ($P > 0.05$) between cultivars for the number of days to symptoms observed in the growth room (Figure 3.3). Symptoms were present by an average of six days post inoculation for every treatment.

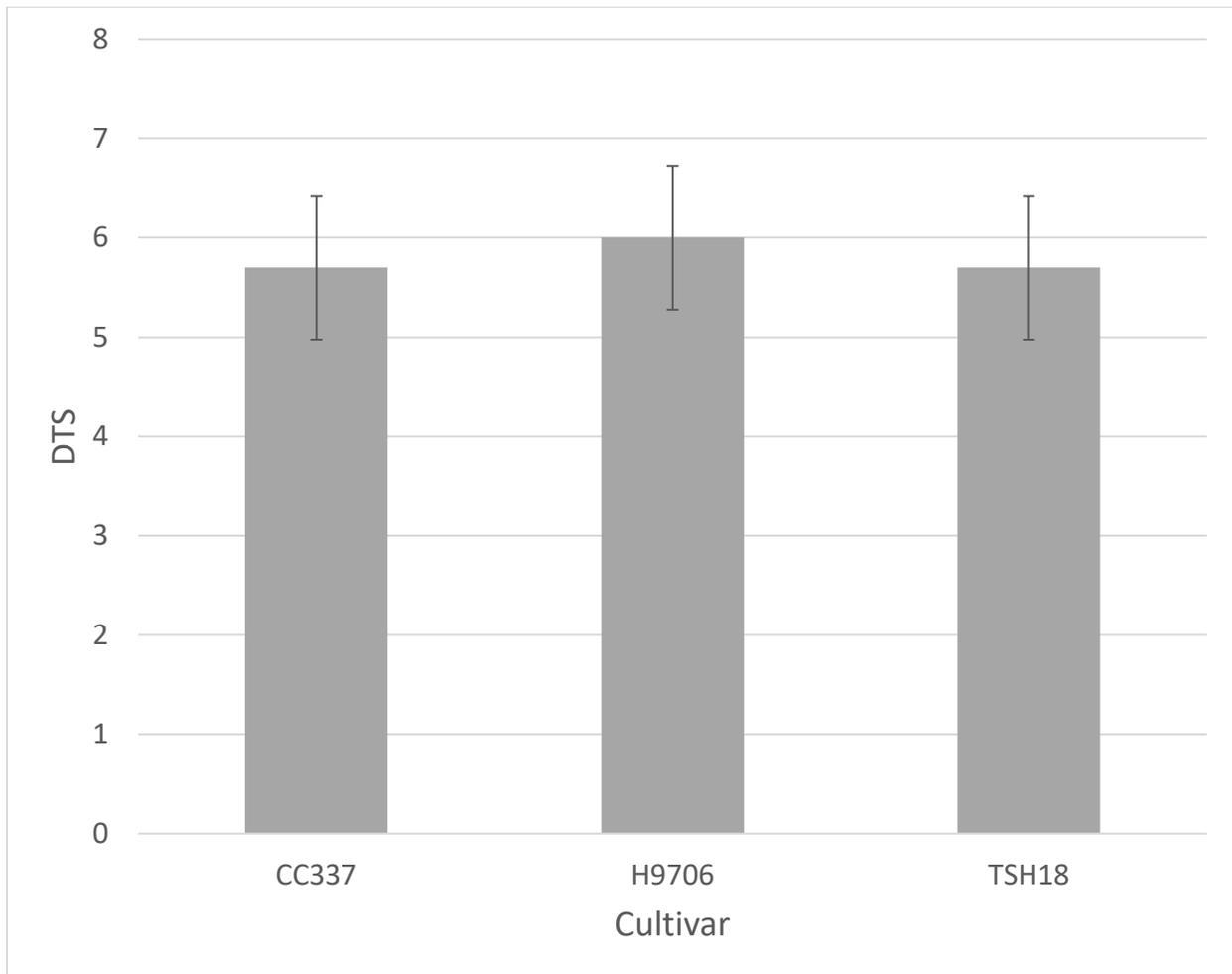


Figure 3-2 Number of days until the appearance of the first bacterial spot symptoms (DTS) after foliar inoculation with *X. gardneri* in a growth room.

Five 25 cell trays were seeded with each cultivar and grown in the Ridgetown Campus greenhouse before being moved to the growth room and inoculated. After inoculation plots were visually inspected daily until symptoms were confirmed by re-isolation and PCR. Data were pooled from two trials completed at the University of Guelph, Ridgetown ON, 2019. Error bars represent standard error of the mean, processed on the normal scale. Bars without letters are not significantly different at $P \leq 0.05$, Tukey's HSD.

3.4.2 Cultivar fruit resistance

3.4.2.1 Field results

Tomato fruit abortion rate ranged from 48 to 72% when flower clusters were inoculated, and 20 to 44% when fruit clusters were (Table 7.2, Appendix 2). Whether clusters were sprayed with bacterial solution or sterile distilled water did not affect the abortion rate in these trials. No differences were found in the DSI when inoculated fruit clusters were evaluated, regardless of growth stage at inoculation (Table 3.5). There was no difference between cultivars in the DSI of fruit that was inoculated at the young fruit stage. The incidence of spots in clusters inoculated at the flower stage differed only between cultivars 'TSH18' (0.1%), 'H3406' (40%) and 'H1178' (36%).

Evaluation of 100 random fruit samples revealed differences in DSI between cultivars 'TSH28', 'Hypeel 696', 'N3306' and 'H9706' with the first being 82% lower than 'H9706' (Table 3.6). Cultivar 'H9706' also had a higher incidence of "Not split" lesions than 'N3306' (81% lower), and 'Hypeel 696' (76% lower), but 'Hypeel 696' did not vary from any other cultivars. When "Small" lesions were assessed, the incidence was 60 to 64% higher in cultivar 'H3406' than in 'Hypeel 696', 'CC337', 'TSH18' and 'TSH28'.

No symptoms were found to have developed on holdback plants that had been incubated for two weeks in the Vegetable Research Facility.

Table 3.5 Overall disease incidence and severity index (DSI) of tomato bacterial spot when reproductive clusters in nine tomato cultivars were target inoculated with *X. gardneri* at the flower or fruit stage (n=4 clusters/type/treatment/year). Cluster harvest data was pooled from three fruit resistance field trials completed at the University of Guelph, Ridgetown ON, 2016-2018.

Cultivar	Flower inoculation ^{a, b}					Fruit Inoculation ^{a, b}				
	Fruit ^c	Incidence (%) ^d	SE ^e	DSI ^f	SE	Fruit ^g	Incidence (%)	SE	DSI	SE
CC337	145	3.9 ab ^b	1.81	0.9 a	1.67	242	0.5 a	2.64	0.2 a	2.45
N3306	108	0.3 ab	1.81	0.1 a	1.67	199	1.7 a	2.64	0.6 a	2.45
H5108	102	7.0 ab	1.81	2.3 a	1.67	240	0.7 a	2.64	0.3 a	2.45
H9706	134	6.9 ab	1.81	2.5 a	1.67	198	0.4 a	2.64	0.3 a	2.45
H1178 ^h	113	35.8 a	1.85	5.7 a	1.71	179	0.4 a	2.66	0.2 a	2.47
Hypeel 696	124	2.1 ab	1.81	0.6 a	1.67	235	16.8 a	2.64	4.8 a	2.45
H3406	115	40.0 a	1.81	7.4 a	1.67	224	1.0 a	2.64	0.4 a	2.45
TSH28	108	18.4 ab	1.81	4.0 a	1.67	214	0.3 a	2.64	0.2 a	2.45
TSH18	104	0.1 b	1.81	0.0 a	1.67	229	2.9 a	2.64	1.4 a	2.45
P-Value		0.0127		0.0322			0.2243		0.2729	

^a Data and standard errors processed on logscale and back transformed using ILINK statement.

^b Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD.

^c The total number of fruit recovered from clusters inoculated at the flower stage, summed over three trials (2016-2018).

^d The percent of cluster inoculated fruit with; any bacterial spots, spots larger or smaller than 5 mm, "Split" or "Not split".

^e SE = standard error of the mean.

^f DSI is the relative weight of the number of lesions, calculated as;

$$DSI = \frac{\sum [(class\ no.) (no.\ of\ fruit\ in\ each\ class)]}{(total\ no.\ fruit\ per\ sample) (no.\ classes - 1)} \times 100$$

^g The total number of fruit recovered from clusters inoculated at the fruit set stage, summed over three trials (2016-2018).

^h Treatment missing one plot in 2018 due to a shortage of transplants.

Table 3.6 Incidence of all Any, small (<5 mm), large (≥5 mm), “Split, and “Not split” bacterial spot lesions on fruit, and associated disease severity index (DSI) for bacterial spot, on 100 red fruit randomly subsampled from a 2 m section of twin-row in nine tomato cultivars target inoculated with *X. gardneri* at the reproductive clusters in two inoculation timings at the reproductive stage. Data collected from fruit resistance field trials completed at the University of Guelph, Ridgetown ON, 2016 – 2018.

Cultivar	DSI ^d	SE ^e	Incidence (%) ^{a, b, c,}									
			Any	SE	<5 mm	SE	≥5 mm	SE	Not split	SE	Split	SE
CC337	1.0 ab	0.53	3.4 a	1.70	1.8 b	0.74	2.9	1.42	2.7 abc	1.22	3.4 a	1.00
N3306	0.6 b	0.29	2.3 a	1.11	2.2 ab	0.91	1.6	0.70	1.8 c	0.77	2.3 a	0.46
H5108	1.8 ab	0.97	5.1 a	2.56	3.0 ab	1.21	3.8	1.77	4.5 abc	1.96	5.1 a	0.54
H9706	3.4 a	1.82	7.5 a	3.71	3.6 ab	1.50	5.7	2.60	9.5 a	4.67	7.5 a	0.96
H1178 ^f	1.6 ab	0.89	6.1 a	3.02	2.4 ab	0.96	3.1	1.33	4.5 abc	1.96	6.1 a	0.87
Hypeel 696	0.7 b	0.35	2.6 a	1.24	1.9 b	0.73	2.7	1.24	2.3 bc	0.96	2.6 a	0.65
H3406	2.1 ab	1.10	6.4 a	3.12	4.8 a	1.92	4.3	1.77	6.0 ab	2.53	6.4 a	0.43
TSH28	0.6 b	0.34	3.2 a	1.57	1.7 b	0.69	2.0	0.88	3.0 abc	1.32	3.2 a	0.63
TSH18	1.2 ab	0.61	3.8 a	1.86	1.8 b	0.71	2.8	1.15	3.3 abc	1.38	3.8 a	0.70
P-Value	0.0020		0.0157		0.0062		0.2890		0.0031		0.8980	

^a The percent of a 100 fruit random sample with any bacterial spots, spots larger or smaller than 5 mm, “Split” or “Not split”.

^b Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey’s HSD

^c All data and standard errors processed on the lognormal distribution to meet normality assumptions and back transformed using ILINK.

^d DSI is the relative weight of the number of lesions, calculated as;

$$DSI = \frac{\sum [(class\ no.) (no.\ of\ fruit\ in\ each\ class)]}{(total\ no.\ fruit\ per\ sample) (no.\ classes - 1)} \times 100$$

^e SE = Standard error of the mean.

^f Treatment missing one plot in 2018 due to a shortage of transplants.

3.4.2.2 Greenhouse results

Only eight symptomatic fruits were recovered from the greenhouse trial, with at least one symptomatic fruit was recovered from each cultivar (data not presented). No symptomatic fruits were recovered from clusters inoculated with sterile distilled water. Abortion rates were calculated to be 35-48% depending on cultivar but there were no differences in abortion rate between clusters inoculated with sterile distilled water or bacterial solution, for any cultivar (Appendix 3).

3.4.3 Relationship among foliar and fruit disease variables

A wide range of correlations were found between measured foliar and fruit variables, but all failed to meet the criteria for a significant and strong positive or negative relationship (Appendix 3).

3.5 Discussion and conclusions

The objective of this study was to compare assessment strategies for resistance in commercial processing tomatoes in Southwestern Ontario to bacterial spot caused by *X. gardneri*. Variables that were considered easier for breeders to assess, such as the days to foliar symptoms, or foliar sAUDPC, were assessed in parallel with fruit quality measurements, which are more laborious to obtain.

Based on the results from the cultivar resistance trials, variables associated with foliar disease severity did not positively correlate with fruit symptom development (Table 4.3, Appendix 3). This supports the discrepancy illustrated by several groups who have attempted to find other treatment options for bacterial spot, especially when looking at organic treatments, where a positive outcome on foliage did not translate to an increase in healthy fruit (P. A. Abbasi et al. 2002; Al-Dahmani et al. 2003; Obradovic et al. 2004; Ji et al. 2006).

The days to symptoms showed no difference between the nine test cultivars, which agreed with growth room validation trials. The days to symptoms data did not predict the differences found in the incidence and severity of fruit symptoms in the foliar trials (Figure 3.1, Table 3.1, Figure 3.3). Days to symptoms is not generally reported in the literature. The sAUDPC for each cultivar also offered limited insight, separating only 'TSH18' from 'H3406', 'Hypeel 696' and 'H9706'. These distinctions were not found in the fruit disease measurements (Figure 3.1, Table 3.1). When percent foliar disease was assessed by DBH timeframes, 'Hypeel 696' had less disease than 'TSH18' and 'H1178' at 20 to 37 DBH, and at 4 to 17 DBH lower than 'TSH18' and 'H1178,' showing

only the cultivars at the two extremes ('Hypeel 696' and 'TSH18') were consistently identified by foliar variables (Figure 3.2). These defoliation results did not consistently translate to the severity of symptoms that would develop on the fruit of the same cultivar. This is consistent with the literature where AUDPC in foliage was a commonly reported variable, and tended to show differences between treatments, yet fruit disease was not similarly impacted (Abbasi et al. 2002; Al-Dahmani et al. 2003; Obradovic et al. 2004).

Abbasi et al. (2002) showed this discrepancy with AUDPC when they were applied to foliage at two different compost rates, they found foliar AUDPC doubled compared to that of the control, but at harvest plants treated with compost treated had fewer or the same percent of fruit with bacterial spot symptoms. The best example in this data set concerns 'Hypeel 696' which had a lower foliar sAUDPC than 'TSH18' but was not different from 'TSH18' in either fruit spot severity or DSI ratings found on fruit in both sets of trials. 'Hypeel 696' was also equivalent in sAUDPC to 'H9706' and 'H3406', none of which differed from TSH18 when fruit were evaluated, indicating the sAUDPC has little predictive power when it comes to fruit health. In the 100-fruit random plot samples of the fruit inoculated trial, 'H3406' presented higher incidence rates of "Small" lesions than 'TSH18' (Table 3.6), and a higher overall incidence from flower-stage inoculated fruit than 'TSH18' (Table 3.5), something not deducible from the sAUDPC values. It seems unlikely defoliation or sAUDPC are predictive for fruit health, supporting the theory that they should not be relied on as the sole indicator of resistance in breeding programs.

Fruit health may then need to be directly assessed, which is a difficult task. Our work used 100-fruit random samples to make data collection meaningful but manageable, compared to whole plant harvests, which should be more useful for breeders assessing novel cultivars (Abbasi et al. 2002; Al-Dahmani et al. 2003; Obradovic et al. 2004). To that end, in our nine cultivars, fruit severity variables were not able to distinguish a single cultivar with resistance to *X. gardneri*, but because resistant cultivars are not commercially available, and experimental cultivars were not included in these trials, this is not entirely unexpected. Regardless, we believe such an approach must be integrated into resistance breeding to produce a truly resistant cultivar.

In our 100 fruit random samples collected from the foliar trials cultivars 'CC337' and 'TSH18' could sometimes be differentiated from one another using fruit severity measurements such as total incidence, incidence of "Large" lesions, and "Not split" lesions, but neither cultivar was different from the remaining ones (Table 3.3). From the DSI values in the same data set, 'N3306' was also statistically equivalent to 'CC337' and 'TSH18', but lower than 'H9706', which was itself higher than 'CC337' and equivalent to 'TSH18,' demonstrating the inconsistency between the different measurements. 'N3306' was in fact equivalent to 'CC337' in all fruit measurements taken from the foliage trials but could only be separated from any other cultivars in two of the six measurements. We believe this emphasizes the importance of the more laborious fruit assessments over foliar data, there does not seem to be sufficient predictive power to disregard one for the other.

When the fruit measurements from this data set were reassessed as a percent of diseased fruit from each cultivar there were no differences between the cultivars (Table 3.4), which is in-line with the foliar assessments, but does not assist in selecting one cultivar over another. Spot severity measurements taken from the 100-fruit random sample of the fruit trial may better reflect the natural infection process, as it required the bacteria to spread plant-to-plant, unlike foliar inoculation, but results in fewer symptomatic fruit overall because of the limited time for the disease to progress.

Finally, there are significant pieces of knowledge missing that prevent the dismissal of any of these variables as assessment tools. The rate of abortion when reproductive clusters were handled was high, 20.6 - 72% depending on cultivar and inoculant (Table 4.2, Appendix 3), unfortunately the basal level of flower abortion is highly variable in tomatoes and related species, and specific basal abortion rates for tomato are not known (Wubs et al. 2007). The natural rate of abortion of untouched clusters was not measured, so the impact of applying liquid to the reproductive clusters as part of the inoculation process on the abortion rate, and in turn on the results, is not known. But given how sensitive reproductive flowers are to mechanical interference it seems likely there was an effect on the observed incidence rates in both field and greenhouse trials. The method of fruit infection by *X. gardneri* is also unknown but, based on these findings; fruit assessments should not be cast aside in favour of foliar-only methods.

One question remains to affect breeder efforts to counter this pathogen, how much influence does genotype have in the face of unpredictable weather conditions? Since the fruit validation trial did not yield conclusive results that question can be only partially

addressed. It is possible that the fruit validation trial did not manifest as expected because the environment was relatively stagnant in the greenhouse, with air movement controlled for temperature regulation, not the abrasive environment of the field, but foliar bacterial spot trials are commonly completed in the facility and foliar symptoms were seen in the validation trial. This supports the conclusion that there is a component of the infection cycle we do not understand and did not properly support.

Meanwhile the field data, collected over three years with variable environmental conditions still passed the COVTEST to be grouped together, indicating the effect of different weather conditions was not so big as to be disqualifying. Figure 4-5 in Appendix 2 illustrates the temperature and precipitation trends over all three field seasons, but the most important periods to determine disease impact are at planting, from approximately mid May – mid June and bloom, from late June – late July. During these critical time periods each trial year was different, May and June 2016 saw less rain than 2017 or 2018 (87mm, 189.5mm and 149mm, respectively) while in 2018 and 2016 more rain fell during July when the plants would be blooming (90.7mm and 79.2mm verses 36.1mm in 2017), and 2018 was the warmest July of the three. Weather is a very important factor in disease outbreak, but statistically it was treated as a random effect, factored in with other variables (ex. Location of the trial) that constituted the year a trial was completed in, leaving some noticeable differences between cultivars which did not have breeder identified resistance to the pathogen. Differences between cultivars like ‘CC337’ and ‘TSH18’ may reflect more their status as late or early maturing cultivars, respectively, than any latent resistance to the pathogen. Therefore, these

results are more likely to be due to differences between cultivars, instead of being strictly weather-related.

4 General Discussion

4.1 Research Justification

Of the four species of Xanthomonads which cause bacterial spot of tomatoes, *Xanthomonas gardneri* is the dominant species in Southwestern Ontario. It is the most problematic due to its adaptability and relative aggressiveness; traits which may be reflective of its relatively distant relation to other tomato BSX (Jones et al. 2004; Abbasi et al. 2015; Timilsina et al. 2019). Copper-based sprays have been the primary method of control since the 1980s, with few effective alternatives or tank mixes resistance in *X. gardneri* has recently rendered copper completely ineffective and they are no longer recommended by OMAFRA (Abbasi et al. 2015; Trueman 2015; Trueman and LeBoeuf 2015).

Since the development of effective controls for this disease remains stagnated, assessment of the effectiveness of alternative methods such as sanitation is paramount. To establish a baseline for the practical use of sanitation, the impact that no sanitation has on pathogen movement during target activities must be established. Work to assess the impact of contaminated implements on the spread of any plant pathogens has been largely limited to direct contact implements such as pruners, or indirect by irrigation water after the plants are planted, neglecting the activities in the planting phase (Baysal-Gurel et al. 2015; Li et al. 2016; BCMA 2018). In the first objective of these studies, the impact of normal activities, such as watering transplants in a trailer (indirect contact) and planting with a transplanter (direct contact), on *X. gardneri* transmission were assessed.

Host resistance is also a commonly exploited method of controlling disease; unfortunately, tomato breeding programs have yet to find sustained resistance to BSX in tomatoes. Unfortunately, there is no single dominant resistance gene to BSX, and, with four causal species to contend with, stable resistance has been elusive. Some experimental lines resistant to BSX have been developed, descending from the cultivar 'Hawaii 7996', but none are commercially available (Whalen et al. 1993; Francis and Miller 2005).

Limited availability of BSX-resistance in available cultivars remains despite years of breeding work and new technologies like genetic markers to help breeders identify and track quantitative (horizontal) resistance genes (Yang and Francis 2005; Foolad and Panthee 2012). Many quantitative genes are required to confer resistance to BSX, which means selecting for them properly is crucial. Many tomato quality assessments are made based on foliar health, which is relatively simple and easy to deduce, but may not be directly related to fruit health (Abbasi et al. 2002; Al-Dahmani et al. 2003; Obradovic et al. 2004; Ji et al. 2006). Yield data may be collected, but fruit quality rarely is, which is problematic due to bacterial spots negative impact on the peelability of the crop, an important quality characteristic (Garcia and Barrett 2006). There have been accounts from the industry that foliar health does not translate to a healthy crop (Dr. C. Trueman, pers. comms.). The second objective of these studies was thus to determine whether foliage assessments accurately forecast fruit quality at harvest.

4.2 Research summary

4.2.1 Sanitation - Trailer

Two replicated trials per year were completed at the University of Guelph, Ridgetown Campus, greenhouse from 2017-2018 to assess the impact the trailer environment could have on pathogen transmission. Placing symptomatic plants at the top of treatment trailers, watering them using one of three common techniques (watering with a hose from the top to the bottom of the trailer, from the bottom to the top or dipping the seedling trays in water for 30 seconds before loading), and closing the trailers overnight resulted in disease symptoms developing throughout the trailer, in every diseased treatment (Figure 2.4). Different watering techniques were assessed to see if a preferred method could be found to limit the impact of the trailer environment before investing in sanitation. When considering distance alone, shelf "A," immediately below the inoculation source, showed more contamination than "C" and "D," 0.8% of plants diseased compared to 0.1 and 0.2% respectively (Figure 2.1, Figure 2.3). All watering techniques facilitated pathogen spread up to the 122.0 cm distance. However, dipping the trays in water caused 90-93% fewer plants to develop symptoms overall, compared to either watering techniques and so should be the recommended watering technique for growers (Figure 2.4). Symptoms were consistently detected 122.0 cm away from the inoculation point, the farthest distance measured. Since symptoms did not develop on holdback plants the symptoms that developed on treated plants can be confidently attributed to the treatments applied in the trailer.

4.2.2 Sanitation – Transplanter

To assess the impact of the transplanter, two trials were completed to assess (1) the transplanter as a vector of transmission, and (2) to assess the importance of this vector compared to the influence of the environment. From 2016 to 2018, two trials were completed to establish the former and three for the latter.

To establish the transplanter as a vector, six symptomatic plants were passed through the implement, followed by 52 healthy seedlings. Every fifth seedling was collected, incubated for 14 days, visually assessed, and then a leaf wash was performed on the combined sample of ten plants. No visual symptoms were found after incubation but colonies of *X. gardneri* were recovered from every diseased plot, regardless of whether plants had been watered before exposure or not (Table 2.1). Variability was too high between trials to combine the two for analysis and within the trials to differentiate the diseased treatments from the clean control. Regardless, disease transmission was consistently found.

In the field, any influence of the transplanter was eclipsed by that of the environment, supporting the idea that the transplanter, while a vector, is not necessarily a major one (Figure 2.5). When the transplanter was used to plant symptomatic seedlings, no pattern in symptom development in the direction of planting was observed indicating that the direction the machine planted had no influence on disease spread. Symptom development followed what was observed in the hand-planted control plots, which used hand-planted symptomatic plants as a positive control.

Environmental patterns over the season were more influential factors than machine direction. In 2016 there was less rain in mid May to mid June than during the same time in 2017 and 2018 (87mm, 189.5mm and 149mm, respectively), while in July the least rain fell in 2017 (36.1mm verses 79.2mm and 90.7mm in 2016 and 2018). Coincidentally 2016 was the only year that the season ended before all plants showed symptoms. Rain during the spring is important for crop health but can also allow a relatively small number of bacteria to become established in a wider area of the field, without the specific direction being of consequence. Planting of these trials occurred during May 30 – June 2 depending on the year, but the first symptoms on non-inoculated plants would not appear until over a month later, as the season progressed into July.

The ease with which the pathogen moved from the inoculated rows into adjacent rows and control treatments is also indicative of environmental influence. Only environmental factors would have facilitated significant east-west movement and inter-treatment movement. This was likely driven by wind and rain in combination, especially during 2017 and 2018 when more rain fell around planting and plants rapidly developed symptoms, however wind direction in the field trials was not measured.

4.2.3 Cultivars – Foliage

Fruit and foliage quality were compared as indicators for BSX resistance in nine commercial cultivars. One trial per year assessed foliage and fruit quality when the

foliage was inoculated, and the other trial when the reproductive structures were targeted. Additionally, verification trials were done in 2018 and 2019 to attempt to replicate results under controlled conditions using three cultivars ('CC337', 'H9706', and 'TSH18') which were noted as showing some variability in the field results.

When the foliage was the target of inoculation, there was no difference between the nine cultivars in the number of days until symptoms developed (Figure 3.1). This was confirmed by validation trials on these cultivars under controlled conditions (Figure 3.3). The sAUDPC was also similar among most cultivars; however, sAUDPC of 'TSH18', an early maturing cultivar, was 52% greater than 'H9706', 'Hypeel 696', and 'H3409'.

When the fruit from these foliar trials was assessed, variability in disease severity index (DSI) was found. The DSI, calculated from the number of lesions on fruit in each cultivar, was lower in 'CC337' than 'H9706' and 'H3406', by 7 and 6 points respectively (Table 3.3). However, 'CC337' had the same incidence of "Any" spots as 'H3406', and only had statistically fewer than 'H9706' and 'TSH18'. Differences were not found between any of the nine cultivars in the incidence of "Small" or "Split" lesions. For "Large" lesions 'CC337' again had a lower incidence than 'H9706' (60%) and 'TSH18' (62%) but also 'H1178' (56%), which it otherwise did not deviate from. For lesions which were "Not split," 'CC337' was equivalent to 'TSH18' but maintained an incidence lower than 'H9706' and 'H3406'. When the incidence of each lesion type was assessed as a percent of diseased fruit in each cultivar no differences between any cultivars were found for any lesion types (Table 3.4).

4.2.4 Cultivars - Fruit

There was a significant abortion of reproductive clusters, after each treatment was applied (Table 4.2, Appendix 3). Disease incidence did not differ between cultivars after targeted inoculation at either the flower or fruit reproductive stage. The DSI of fruit stage inoculated clusters also showed no difference between cultivars, with between 179 and 242 fruit recovered per cultivar from 2016 to 2018. The incidence of bacterial spot on fruit that had been inoculated at the flowering stage was higher in 'H1178' and 'H3406' compared to 'TSH18', which displayed 35.8, 40.0 and 0.1% fruit with spot respectively (Table 3.5).

When random fruit samples were taken from fruit trial plots, 'N3306', 'Hypeel 696' and 'TSH28' had a lower DSI than 'H9706' (Table 3.6). 'H9706' also had a higher incidence of "Small" lesions compared to 'CC337', 'Hypeel 696', 'TSH28' and 'TSH18' under these conditions, but no differences were found between cultivars when "Large" or "Split" lesions were assessed. The only differences in "Not split" lesions between cultivars came between 'H9706 and 'N3306', 1.8% compared to 9.5% respectively.

4.3 Research conclusions

4.3.1 Sanitation

These results demonstrate that the environment within the transport trailer favours disease infection, especially when plants are watered and left sealed in the trailers for extended periods. Transmission of the pathogen in the transplanter occurred at insignificant levels and was not an influence in the field compared to the influence of weather in the field environment. Based on these findings, only one hypothesis

concerning the transmission of the pathogen was accepted, transmission was found to occur, but the significance of that transmission, the conclusions and thus the recommendations drawn from them differ.

Based on the results of different watering techniques that can be used to water seedling trays in a trailer, dipping trays in water for 30 seconds, rather than spraying them, should be recommended. This technique was not able to eliminate the transmission of the pathogen but was superior to either hose technique, with up to 93% reduction in disease incidence compared to irrigating from the bottom to the top of the trailer. There may still be difficulty in persuading the industry to adopt this laborious, expensive and time-consuming technique, but it should be the recommended one for watering plug tray seedlings.

The usefulness of investing the time, money and labour to clean the transplanter should be made on a case by case basis. Because season long forecasts are unreliable predicting outbreak conducive years is difficult, some growers may decide that the moderate impact the transplanter has as a pathogen vector is not worth the investment, while others may not wish to take the chance. This will require products and procedures to be developed for pieces of equipment, but the emphasis should be placed on the trailer.

4.3.2 Cultivars

In this work, there was little correlation between symptom development on foliage and fruit (Table 4.3, Appendix 3). There was no significant difference between cultivars

in the number of days it took for symptoms to develop, and differences found in sAUDPC did not translate to differences in fruit quality. The maturity rate of the cultivar also influences foliar parameters, but by determining the sAUDPC in terms of “Days to Harvest,” rather than calendar dates, the influence of that variable should be minimized.

When looking at fruit collected from foliar inoculated plants, the DSI was not a sensitive enough measurement of fruit health to identify all cultivars that showed extremes in the number or severity of spots. This may indicate DSI may be too broad to show relative fruit health between cultivars. For example, ‘CC337’ showed low DSI and had a low incidence in “Any,” “Large” and “Not split” lesion denominations, but ‘N3306’, which had the same DSI only had a lower incidence of “Not split” lesions. The same trend was found in cultivars with very high DSI. Only ‘H9706’ was flagged with high DSI, but it had a high incidence of “Any,” “Large” and “Not split” lesions, which ‘TSH18’ also exhibited, without the accompanying notable DSI. The same trend of inconsistencies in cultivars flagged by DSI continued in the random sample of the fruit inoculated trial, and no cultivars were flagged at all in the cluster inoculations, despite ‘H1178’, ‘H3406’ and ‘TSH18’ having vastly different incidence values. Thorough evaluations and comparisons between cultivars may thus require such detailed measurements, rather than overview foliar and fruit DSI ratings.

The validation trial of fruit and flower inoculated plants provided only eight symptomatic fruits. A statistical analysis could not be performed, but diseased fruits were recovered from each cultivar, and no symptomatic fruits were recovered from plants inoculated with sterile distilled water. This shows the inoculation method did not

cross contaminate, but because the infection process is unknown it is unclear why diseased fruit were not produced in higher numbers if fruit hairs were the point of entry. The abortion rate of handled reproductive clusters ranged from 35-48% depending on cultivar, similar to rates seen in the field trials.

No foliage parameters consistently predicted the incidence of spots on harvested fruit, supporting the hypothesis that the two are not as closely related as originally believed. Unfortunately, cultivars with resistance specifically to *X. gardneri* have not been developed, and those with resistance to other species of *Xanthomonas* were not commercially available, and therefore not part of this work. Thus, truly positive or negative controls are not available, but the assessment of fruit damage may be necessary to identify such cultivars. 'Ohio 9834' and 'Ohio 9816' are cultivars reported to have partial resistance to *Xanthomonas campestris* pv. *vesicatoria* race T1 (likely *X. euvesicatoria* under Jones 2004), but the resistance reported was based predominantly on foliar evaluations not fruit quality (Jones et al. 2004; Francis and Miller 2005).

Environmental conditions are the other outstanding factor after the presence of the pathogen itself. In the outdoor transmission trials, weather seemed to mask the influence of the transplanter. In the fruit disease trials, the COVTEST completed as part of the statistical analysis showed that random factors, such as 'year' (i.e. environmental conditions, location of the trial ex.) were not so extreme as to require data to be analysed separately from one year to the next. The differences between years included one factor breeders consider very important, a wetter blooming period (late June to late July) in 2018 and 2016 than in 2017 (90.7mm, 79.2mm, 36.1mm in 2017). This seems

to indicate the weather is a changing influence, but the constant influence of cultivar was perceptible, despite not having a 'resistant' genotype. Weather is very influential in spreading existing pathogens and determining the severity of an outbreak, but the initial susceptibility of the plant is related to genotype. Unfortunately, factors related to an optimized genotype remain difficult to utilize or unknown altogether.

Resistant cultivars are needed by the industry but based on the results found here; foliar evaluations should be combined with those from fruit for an improved evaluation of the cultivar. Based on these findings, the hypothesis that fruit symptoms were more variable between processing cultivars than were foliar symptoms was accepted.

4.4 Future research

4.4.1 Sanitation

Seed sanitation procedures must strike a balance between protecting the seed embryo while trying to kill the pathogen within the seed, so while this should remain the first step in the cycle, it is not a complete solution. This means everyone involved in the growing process should treat plants and anything they encounter as potentially contaminated, especially any water that contacts the plants as it can effectively move bacteria from one host to another.

In our work the impact of the trailer on pathogen dissemination was clear, but our approach to establish the transplanter as a vector may have been too broad, and lead to the issues causing inconsistent recovery of the bacteria. Since a specific tissue amount was necessary for each leaf wash, we did not have enough tissue to test individual plants and instead tested the plot. Since the field trial showed that the direction the machine moved in had little effect, the need to sanitize the transplanter likely hinges on just how many plants are infected after a symptomatic plant passes through, which could not be assessed here.

If transmission through the transplanter affects the next five plants the zone of potential outbreak is relatively the same as just having one symptomatic plant, but if 20 plants can be infected it could would broaden the source of the outbreak considerably. Other pathogens such as CMM and CGMMV have shown that transmissibility is highly variable, and more work should be done to understand this with *X. gardneri* (Sharabani et al. 2013; Li et al. 2016). This research should include the development of new in field

detection techniques, such as swab testing, or the reduction of cost and tissue requirements for DNA techniques, such as real-time PCR or leaf washes.

The field transmission trials make clear that, while the transplanter can transmit an insignificant number of the bacteria from one seedling to the next, it is the environmental conditions that have the largest impact on outbreak potential. However, because we cannot see the pathogen and cannot predict what conditions will be in July when planting in May some growers may still prefer to sanitize the transplanter on a routine basis, which will still require best practices be established.

A more in-depth look at the transmission pattern from the transplanter would be needed to identify how many plants the pathogen was passed too after a diseased plant. Such work would require either the development of a leaf wash protocol with lower tissue requirements or a real-time PCR protocol with low tissue requirements. The technique should also be applied to holdback plants to get a better understanding of the potential epiphytic population of the pathogen rather than relying on symptom development. Such work would help growers understand just how large the potential outbreak zone could be, something not achieved in this work. For example, if five plants are infected after a single carrier plant the potential outbreak zone is very different than if 15 plants are affected, such information is needed to truly understand the transplanter's role in a worst-case scenario.

To sanitize the equipment both the canvas material that makes up the plug trailer and the aluminum of the transplanter carousel should be matched with sanitation

products which will not weaken them, yet still be effective against *X. gardneri*. For example alkaline and acidic cleaners will both corrode the aluminum of the transplanter, so a specially designed product like KleenGro, or fast acting quaternary ammonium compounds may be required (Nguyen et al. 2017; PACE 2019; OMAFRA 2019 May 14).

For the trailer, the best watering technique to reduce pathogen spread was dipping the trays and should be the recommended technique, but the extra time and labour involved may make this difficult. Mechanization and automation of dip watering would likely improve feasibility. Dipping trays would not eliminate the need to sanitize the trailer, and both practices should be implemented.

4.4.2 Cultivars

Beyond the implementation of fruit observations into the evaluation of cultivars, more work is required to understand *Xanthomonas gardneri* infection of the tomato fruit. Disease evaluations of fruit are laborious, but the number of variables evaluated can be reduced if the ideal target phenotypes are known, which requires understanding how the bacteria infect the fruit.

Fruit hairs were believed to be the gateway for fruit infection (Horodecka 1989; Wu et al. 2003; Gudesblat et al. 2009b; Zhang et al. 2009; Bhattarai et al. 2016). The failure of the fruit validation trial to produce symptomatic fruit, despite inoculation with the pathogen after disturbing the fruit hair, may contest fruit hairs as the entryway by which fruit disease occurs. The validation trial was not designed to test fruit hairs as the entry point, and the artificial disturbance of the hairs may have had unknown

consequences, but it raises the point that the infection pathway is not clear. Better understanding of the infection process that affects fruit may also help growers understand the relative impact of the weather during the growing season. If the blossom or fruit hair are the primary target, then understanding how invasion is achieved will affect what protections may or may not be viable. For example, if wind damaged fruit hairs are the primary entry point, a tree line or artificial windbreak may be helpful, or perhaps a genotype with stronger hairs, or perhaps no hairs? Such questions cannot be properly answered at this time, because the bacteria's method of infecting fruit remains the critical missing information.

On the other hand, *Salmonella enterica* may provide an unexpected clue to *Xanthomonas* infection of tomatoes. *Salmonella enterica* is often recovered in *X. perforans* infected plants but *S. enterica* infects systemically, making its entrance via the plants roots (Potnis et al. 2014). Systemic infection of plants by *X. gardneri* has not been reported, but the host traits required for resistance to a systemic invader would be drastically different from one invading by surface hairs. Attempts to tag and follow the infection process of *Xanthomonads* are ongoing, and such information will be vital to creating a resistant host and a more holistic disease management action plan (Rotondo 2016).

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APPENDIX 1

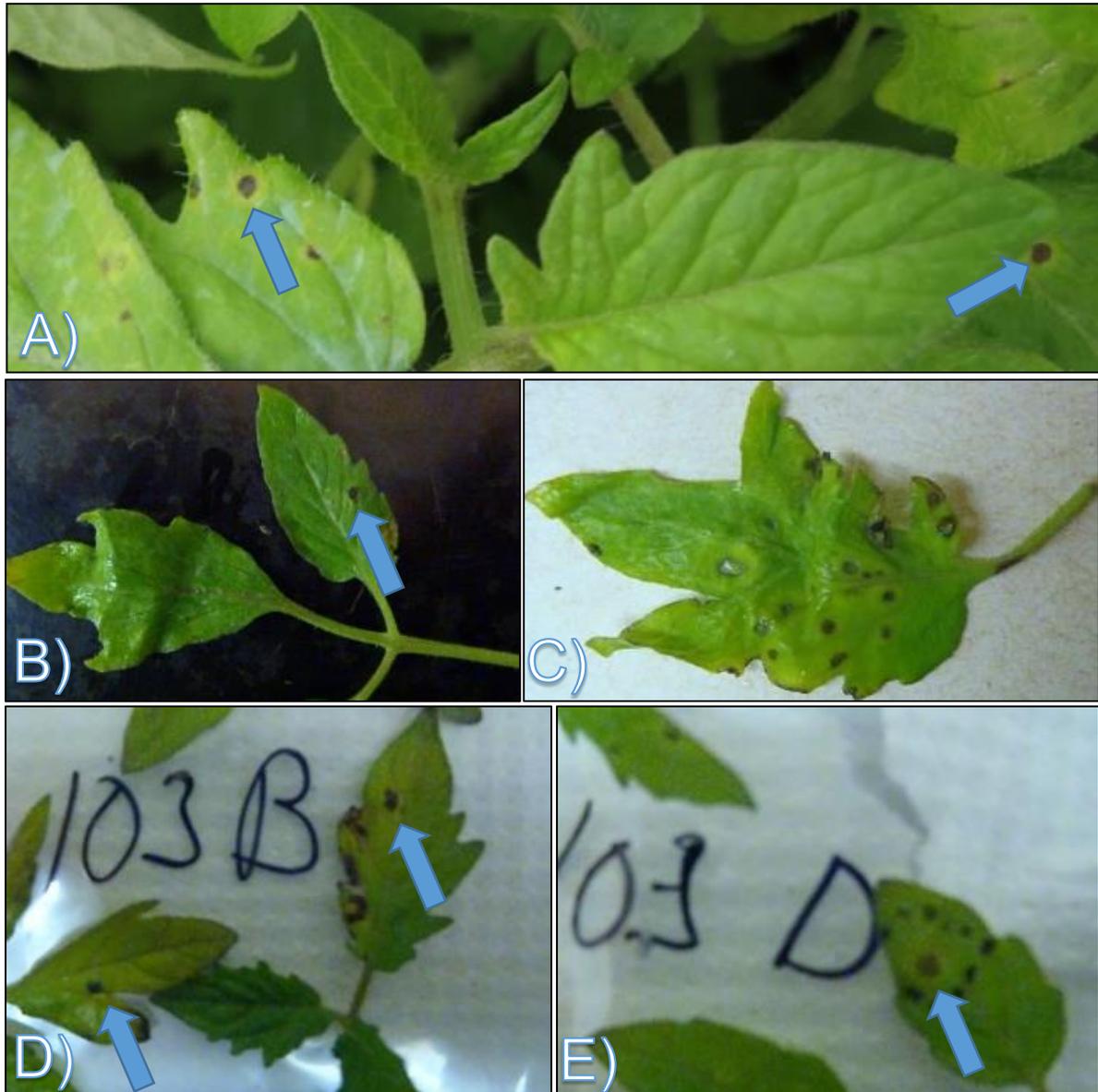
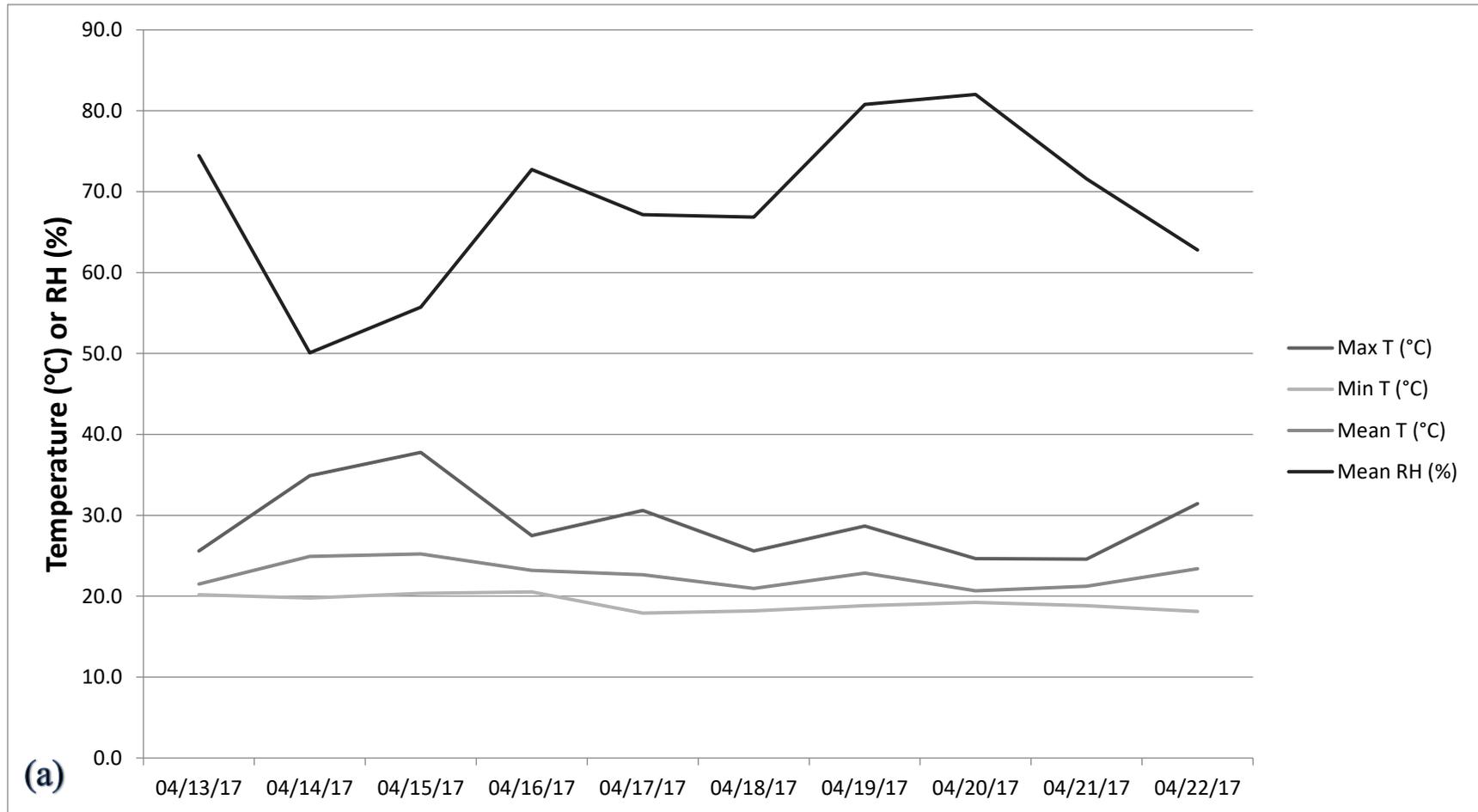


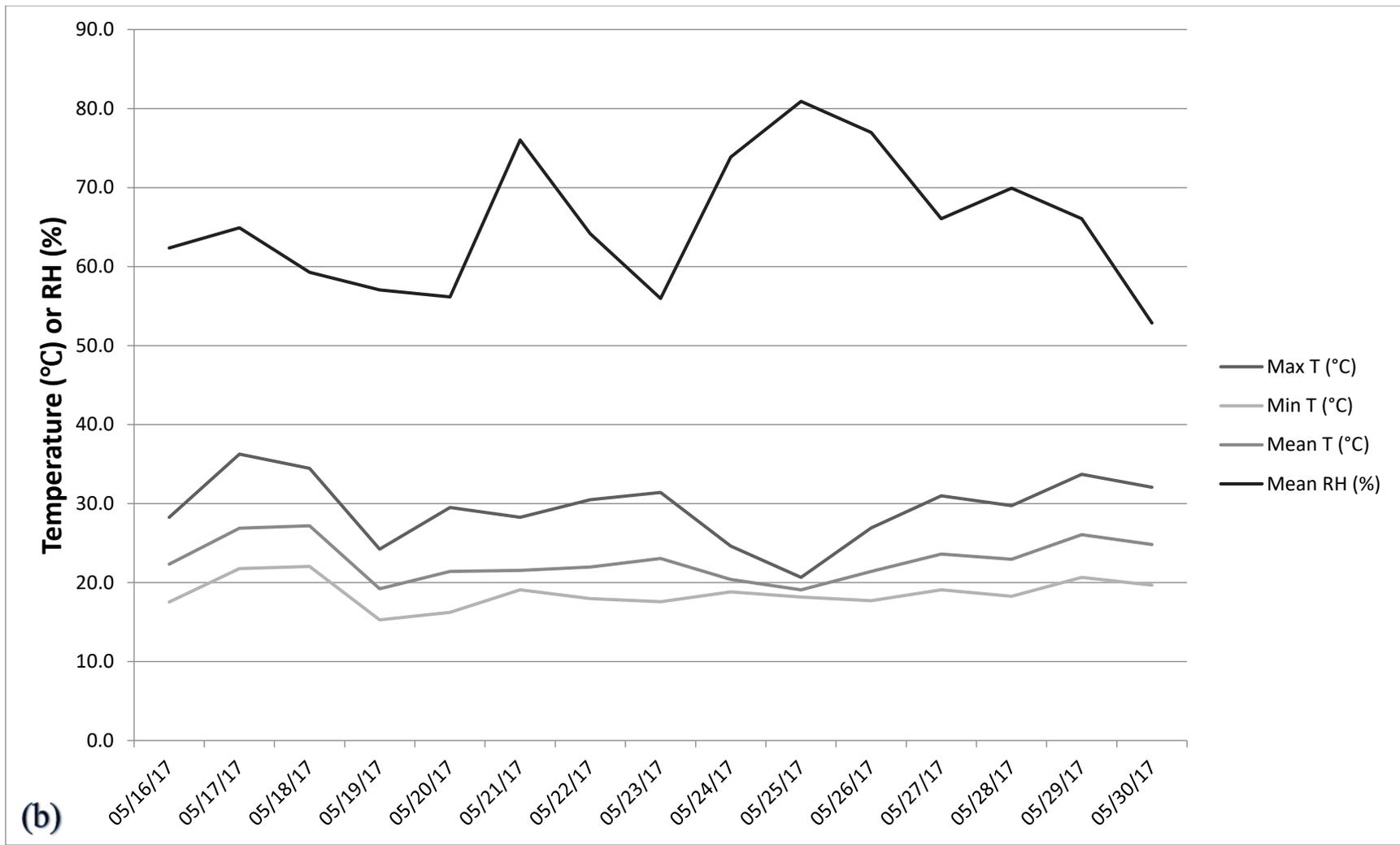
Figure 4-1 Photo key of bacterial spot symptoms used to differentiate symptoms in both field and validation trials between those caused by *X. gardneri* and other diseases.

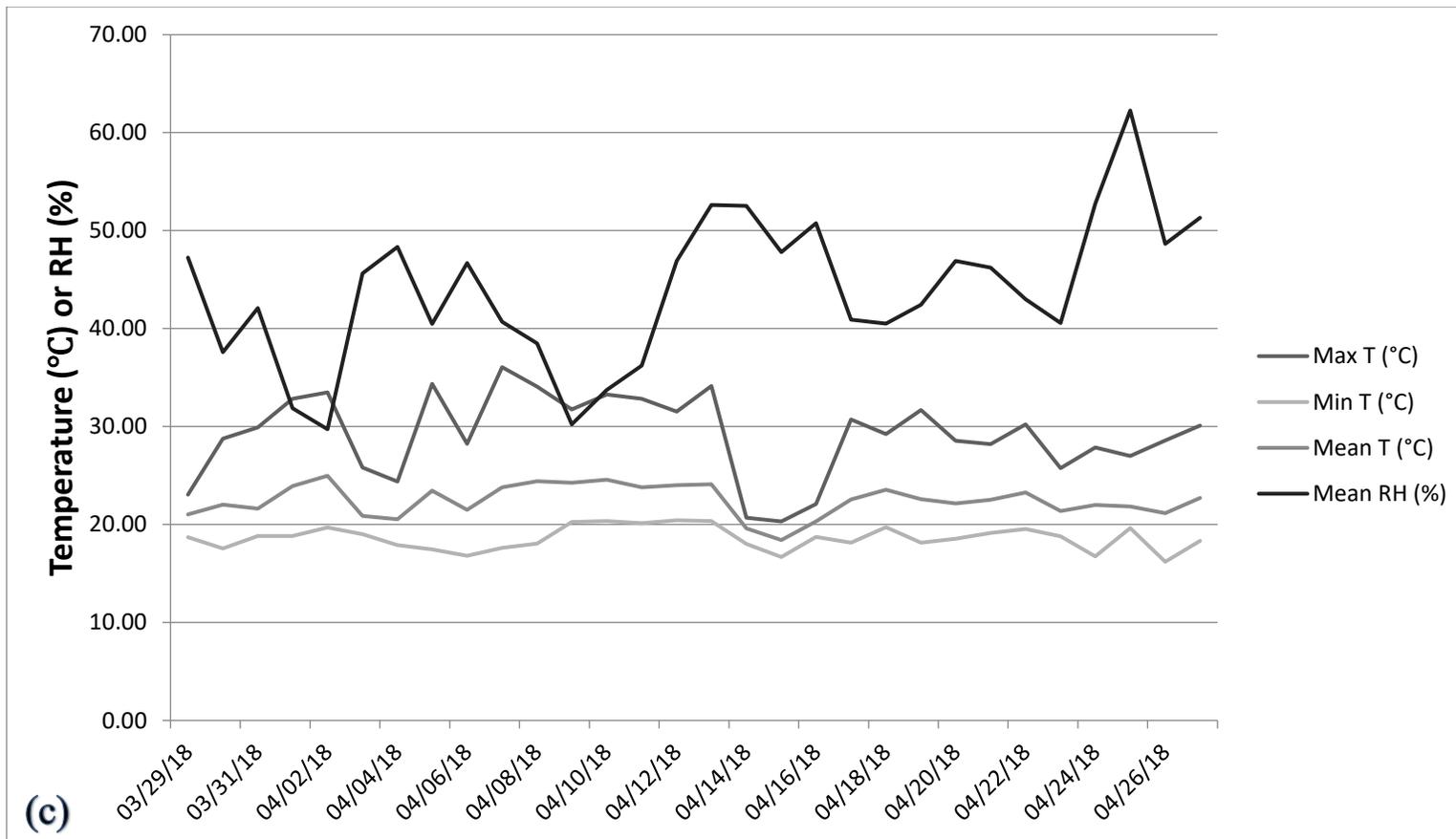
X. gardneri lesions are usually at least 3mm in diameter, brown-grey in colour, with (A) or without (B) yellow chlorosis halo and may appear wet (C). Some examples of unrelated but similarly presenting lesions include; fungal lesions (D) which are larger and irregularly shaped, and other bacterial diseases (E) which have chlorosis but are lighter in colour.

APPENDIX 2



(a)





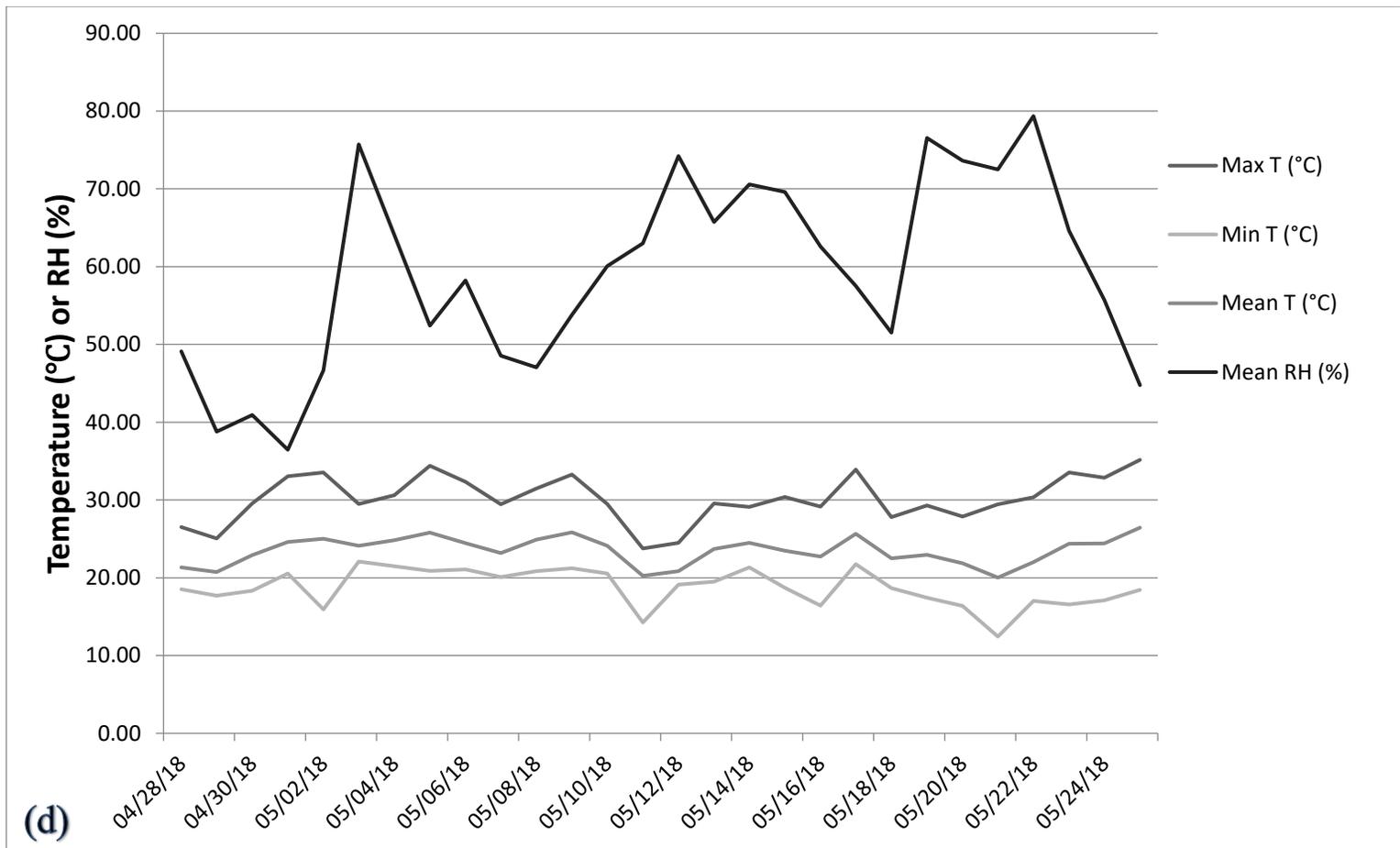


Figure 4-2 Environmental conditions in the Ridgetown Campus greenhouse (canopy level) during trailer dissemination trials in April & May 2017 and 2018.

A) Trial 1, inoculation on April 13 and 14, incubated until harvest 8 days later. B) Trial 2 treatment application on May 16 and 17 and incubated until harvest May 29 and 30. C) Trial 3, treated on April 12 and 13, 2018, and incubated until assessment, April 26 and 27. D) Trial 4, treatments applied on May 10 and 11 and incubated until assessment, May 24 and 25.

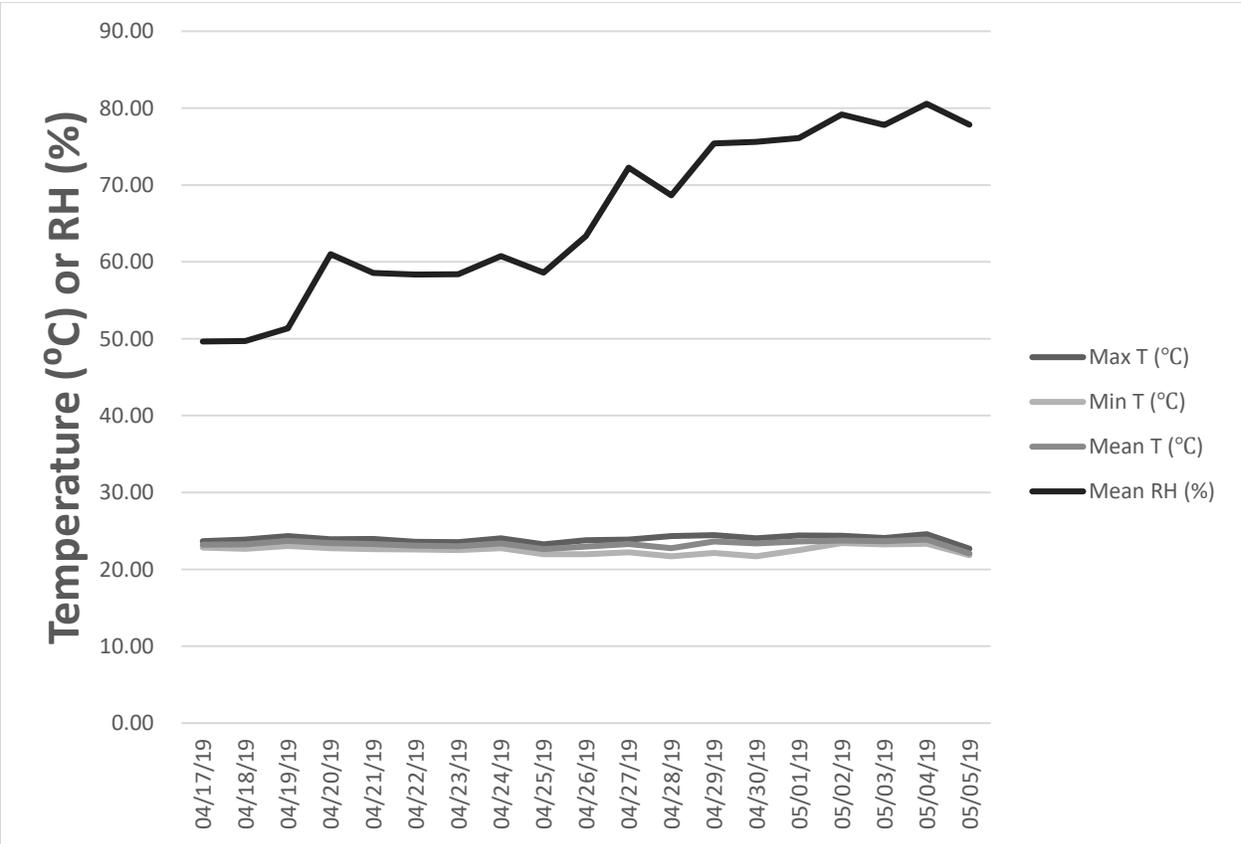


Figure 4-3 Environmental conditions in the Ridgetown Campus growth chamber during completion of the indoor dissemination trial, April & May 2019.
 Environmental data for 2018 trial was unavailable due to a sensor failure.

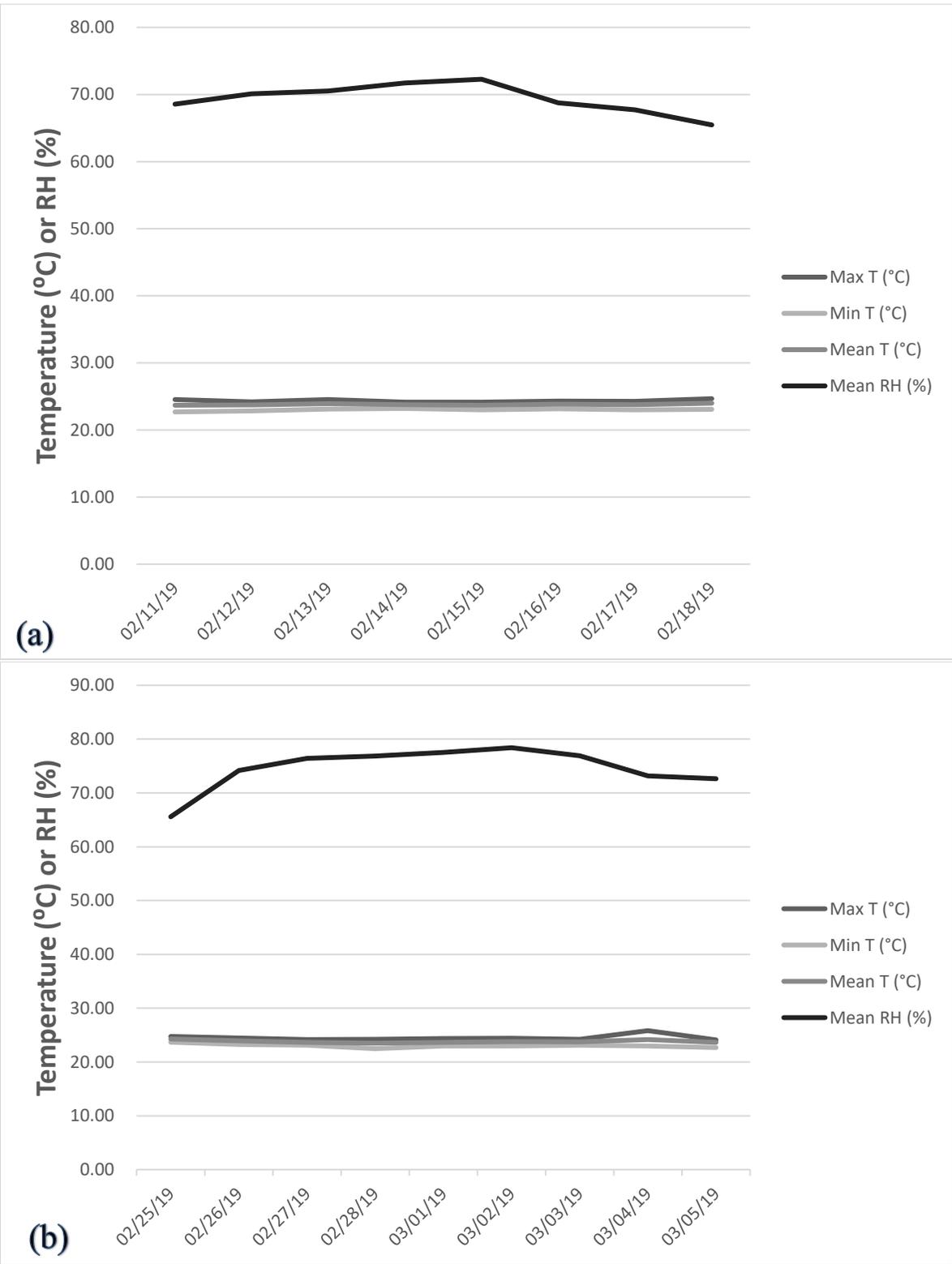
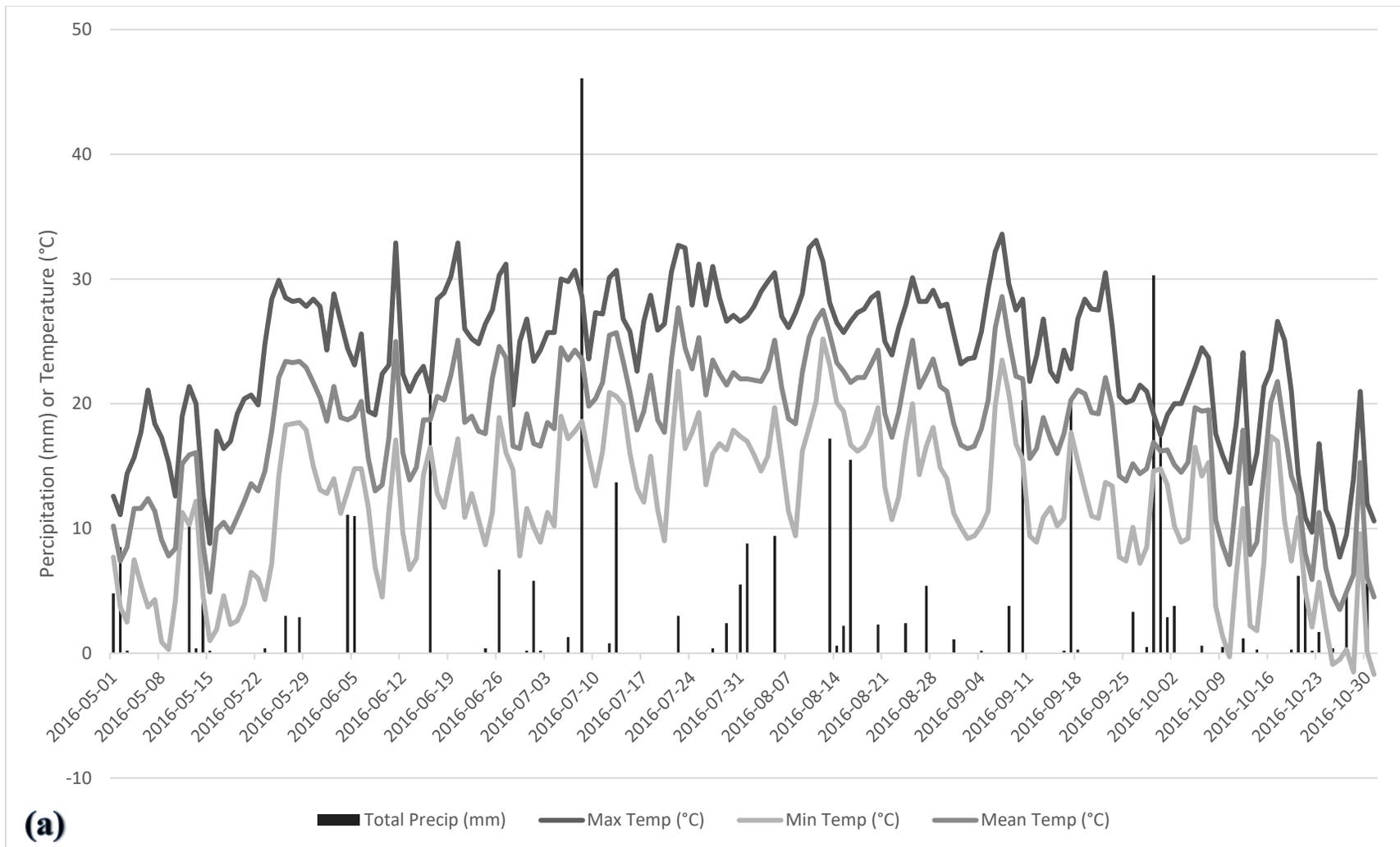
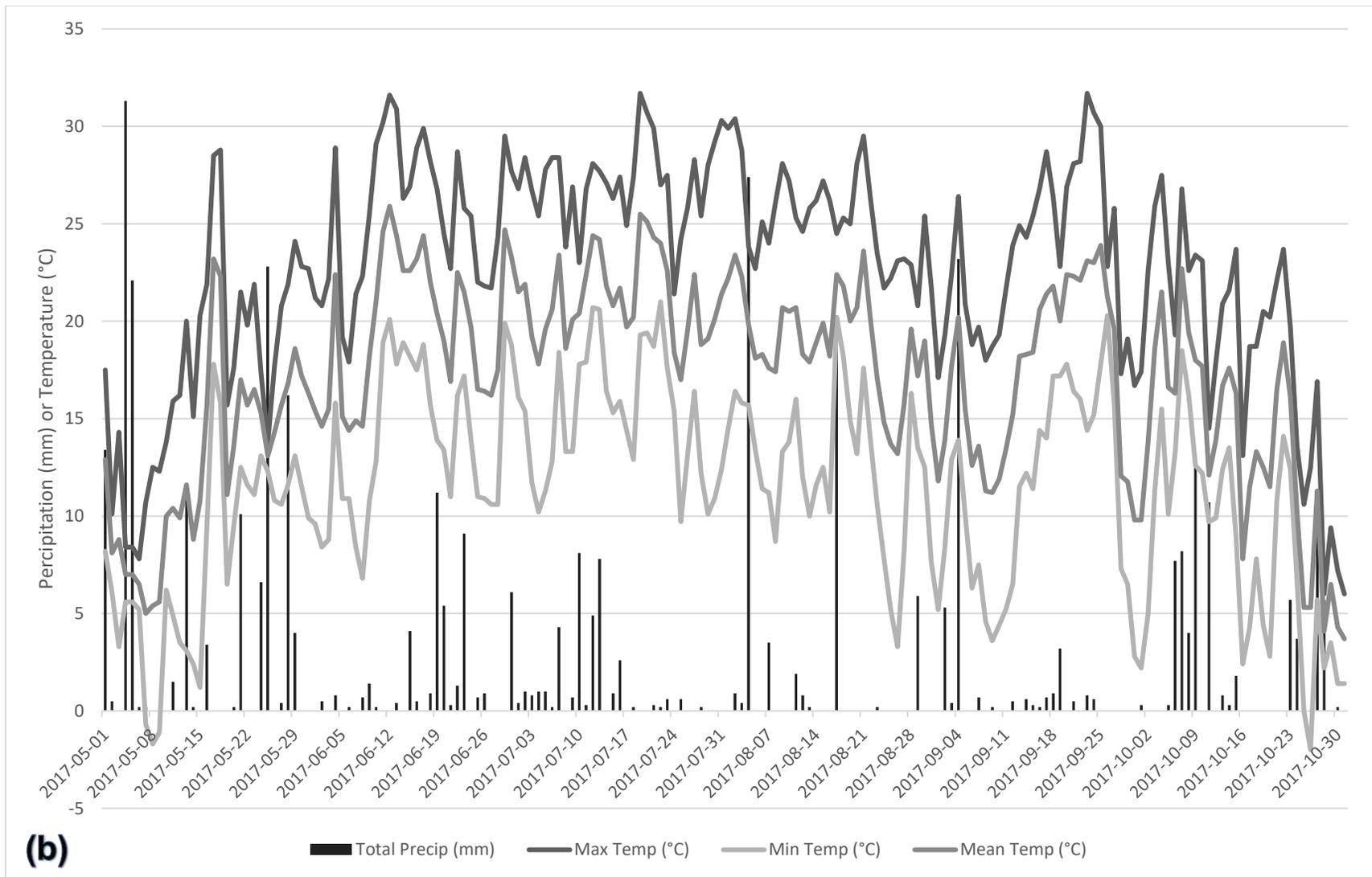


Figure 4-4 Environmental conditions in the Ridgetown Campus growth chamber during completion of foliar resistance validation trials a) Trial 1, and b) Trial 2.





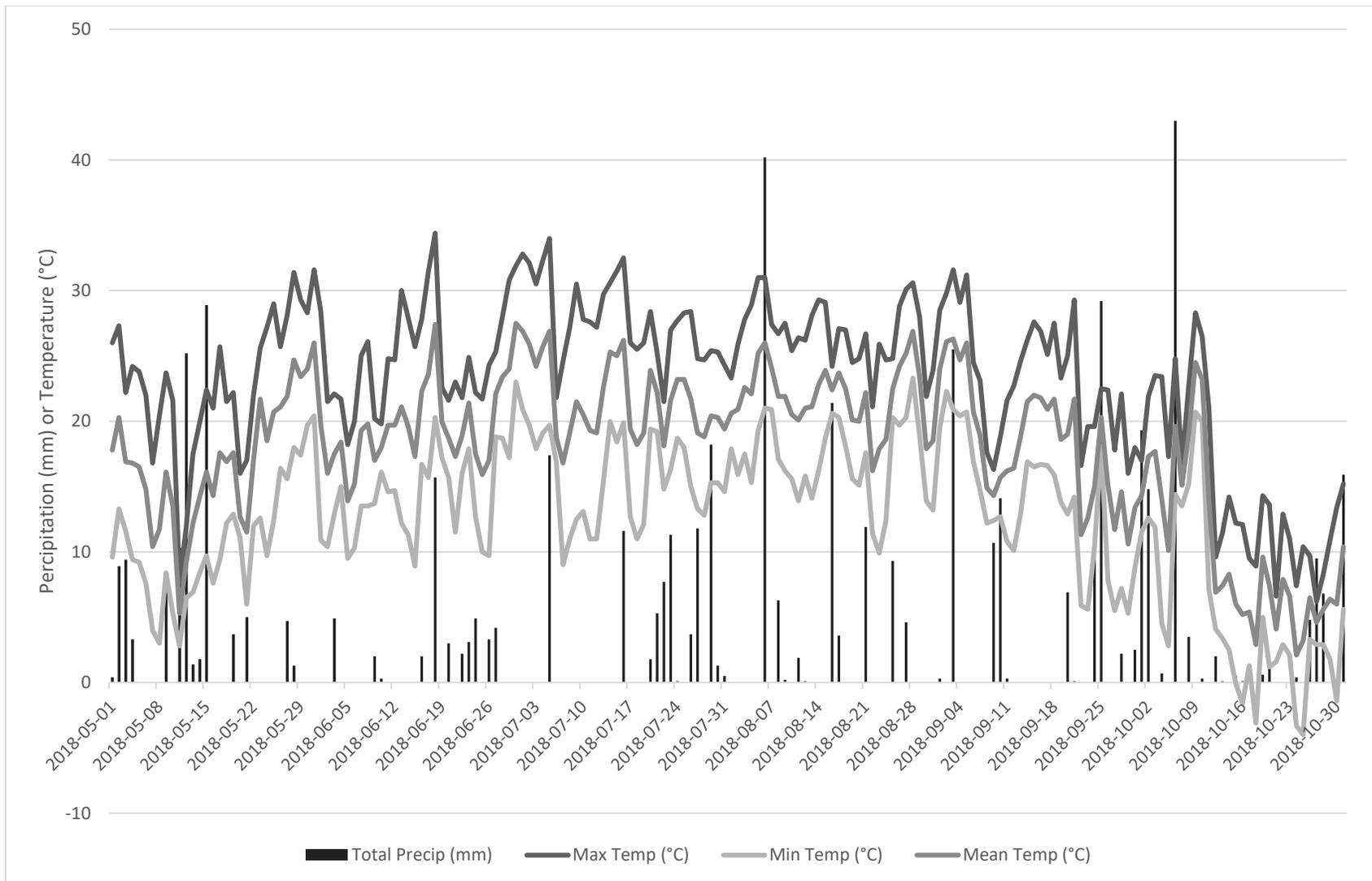


Figure 4-5 Environmental conditions for Ridgetown Campus field trials during completion of all trials during (a) 2016, (b) 2017 and (c) 2018. Data compiled from Environment Canada weather station 71307 RIDGETOWN RCS (42.45°N, 81.88°W).

APPENDIX 3

Table A3.1 Total, red, green and rotten fruit yield in a 2 m section of twin-row in nine tomato cultivars foliar inoculated with *X. gardneri* seven to ten days after transplanting. Data collected from foliar resistance field trials completed at the University of Guelph, Ridgetown ON, 2016-2018.

Cultivar	Yield (kg) ^{a, b}							
	Total	SE ^c	Red	SE	Green	SE	Rotten fruit	SE
CC337	29.2 abc	2.68	24.7 abc	2.03	3.0 a	1.39	0.2 a	0.08
N3306	23.0 d	2.11	20.1 c	1.66	2.0 ab	0.93	0.2 a	0.11
H5108	27.6 bcd	2.54	24.4 bc	2.00	2.4 ab	1.10	0.3 a	0.13
H9706	36.5 a	3.35	32.1 abc	2.64	3.0 a	1.36	0.4 a	0.19
H1178	33.1 ab	3.04	28.3 abc	2.32	3.0 a	1.39	0.4 a	0.20
Hypeel 696	33.7 ab	3.09	28.4 abc	2.34	3.6 a	1.67	0.4 a	0.20
H3406	33.1 ab	3.04	28.9 abc	2.38	3.1 a	1.42	0.4 a	0.19
TSH28	23.2 cd	2.13	19.7 c	1.62	2.3 ab	1.07	0.3 a	0.15
TSH18	21.8 d	2.00	19.5 c	1.61	1.2 b	0.54	0.2 a	0.10
P-Value	<0.0001		<0.0001		0.0033		0.2771	

^a Data processed on the logarithmic scale and back transformed using ILINK.

^b Numbers in a column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HS.

^c SE = standard error of the mean.

Table A3.2 Percent of reproductive units aborted when reproductive clusters were inoculated with *X. gardneri* solution (Inoculated n=4 clusters/type/treatment/year) or sterile distilled water (Control n=10 clusters/type/year). Data pooled from three fruit resistance field trials completed at the University of Guelph, Ridgetown ON, 2016 – 2018.

Cultivar	Aborted (%) ^a							
	Flower Inoculated ^b	SE ^c	Flower Control ^d	SE	Fruit Inoculation ^b	SE	Fruit Control ^e	SE
CC337	54.7 ab	10.67	50.4 a	17.45	31.2 abc	9.06	37.1 a	12.27
N3306	69.8 a	10.67	67.6 a	17.36	43.6 a	9.06	53.1 a	12.85
H5108	67.5 a	10.67	66.5 a	16.80	28.1 bc	9.06	30.5 a	15.18
H9706	59.7 ab	10.67	54.3 a	20.68	20.6 c	9.06	48.9 a	12.85
H1178 ^f	48.4 b	10.75	40.9 a	17.07	27.6 bc	9.11	36.8 a	13.02
Hypeel 696	63.1 ab	10.67	50.7 a	17.36	29.6 abc	9.06	58.4 a	15.18
H3406	61.7 ab	10.67	47.2 a	20.68	41.2 ab	9.06	35.9 a	11.14
TSH28	72.0 a	10.67	48.5 a	18.37	37.0 ab	9.06	45.7 a	11.15
TSH18	66.8 ab	10.67	66.9 a	18.37	43.6 a	9.06	53.3 a	11.14
P-Value	0.0028		0.4098		<0.0001		0.4575	

^a Numbers in a column followed by the same letter are not significantly different at $P \leq 0.05$, Tukey's HSD, data and standard errors processed on the normal scale.

^b Number of fruits recovered from flower or fruit stage reproductive clusters inoculated with *X. gardneri* are given in Table 3.4.

^c SE = standard error of the mean.

^d Total fruit recovery from flower stage, water inoculated control clusters, summed over three years, ranged from 14 to 34 fruit.

^e Total fruit recovery from fruit stage, water inoculated control clusters, summed over three years, ranged from 13 to 24 fruit.

^f Treatment missing one plot in 2018 due to a shortage of transplants.

Table A3.3 Spearman's ranked correlation (r_s) coefficients for foliar standardized area under the disease progress curve (sAUDPC) and the incidence of fruit symptom variables measured in nine tomato cultivars foliar inoculated with *X. gardneri* seven to ten days after transplanting in foliar resistance field trials completed at the University of Guelph, Ridgetown ON, 2016-2018.

Cultivar		sAUDPC ^a x DSI ^b	sAUDPC x Any	sAUDPC x <5 mm	sAUDPC x ≥ 5 mm	sAUDPC x Not Split	sAUDPC x Split
All	r_s	0.01 ^c	-0.02	0.15	0.01	0.22	-0.02
	p-value	0.9647	0.8728	0.1220	0.8854	0.0223	0.8690
CC337	r_s	-0.32	-0.32	-0.08	-0.33	-0.13	-0.30
	p-value	0.3126	0.3061	0.7940	0.2906	0.6876	0.3515
N3306	r_s	0.28	0.35	0.05	0.27	0.47	0.19
	p-value	0.3839	0.2643	0.8873	0.3949	0.1225	0.5486
H5108	r_s	-0.32	-0.31	0.27	-0.48	-0.43	-0.21
	p-value	0.3079	0.3230	0.3995	0.1121	0.1667	0.5193
H9706	r_s	0.06	0.20	-0.32	0.26	0.39	0.08
	p-value	0.8542	0.5339	0.3070	0.4094	0.2063	0.7951
H1178 ^d	r_s	-0.71	-0.68	-0.16	-0.52	0.12	-0.66
	p-value	0.0102	0.0158	0.6092	0.0800	0.7129	0.0185
Hypeel 696	r_s	0.37	0.33	0.44	0.11	0.50	0.12
	p-value	0.2356	0.3006	0.1509	0.7287	0.0952	0.7038
H3406	r_s	-0.20	-0.22	0.01	-0.21	0.20	-0.10
	p-value	0.5419	0.4845	0.9828	0.5128	0.5339	0.7530
TSH28	r_s	-0.11	-0.19	0.39	-0.27	0.04	-0.25
	p-value	0.7292	0.5567	0.2116	0.3902	0.8970	0.4291
TSH18	r_s	0.36	0.43	0.72	0.43	0.64	0.51
	p-value	0.2453	0.1667	0.0077	0.1634	0.00261	0.0877

^a sAUDPC is calculated from defoliation values for each cultivar using their respective DBH defoliation values where Y_i is percent defoliation at day X_i , and Y_{i-1} is percent defoliation at day X_{i-1}

$$\text{AUDPC} = \sum [((Y_i + Y_{i-1}) (X_i - X_{i-1}))/2] \text{ standardized for the length of assessment period becomes:}$$

$$\text{sAUDPC} = \text{AUDPC}/\text{number of days}$$

^b DSI is the relative weight of the number of lesions, calculated as;

$$\text{DSI} = \frac{\sum [(\text{class no.}) (\text{no. of fruit in each class})]}{(\text{total no. fruit per sample}) (\text{no. classes} - 1)} \times 100$$

^c *Rs* values in the first row reflect the relationship between variables summed over all treatments

^d Treatment missing one plot in 2018 fruit trial due to a shortage of transplants.

Table A3.4 Spearman's ranked correlation (r_s) coefficients for foliar standardized area under the disease progress curve (sAUDPC) measured in nine tomato cultivars foliar inoculated with *X. gardneri* seven to ten days after transplanting in foliar resistance field trials, and the incidence of fruit symptom variables from 100 red fruit randomly subsampled from a 2 m section of twin-row in nine tomato cultivars target inoculated with *X. gardneri* at the reproductive clusters in two inoculation timings at the reproductive stage and sorted by foliar trial randomization. Foliar and fruit resistance trials completed at the University of Guelph, Ridgetown ON, 2016-2018.

Cultivar		sAUDPC ^a x DSI ^b	sAUDPC x Any	sAUDPC x <5 mm	sAUDPC x ≥ 5 mm	sAUDPC x Not Split	AUDPC x Split
All	r_s	0.27 ^c	0.32	0.25	0.34	0.29	0.25
	p-value	0.0047	0.0007	0.0081	0.0003	0.0025	0.0108
CC337	r_s	0.17	0.24	0.02	0.26	0.14	0.16
	p-value	0.6006	0.4520	0.9615	0.4222	0.6747	0.6189
N3306	r_s	0.05	0.12	0.10	0.22	0.01	0.01
	p-value	0.8707	0.7099	0.7473	0.4970	0.9738	0.9643
H5108	r_s	0.14	0.30	0.37	0.34	0.30	0.40
	p-value	0.6670	0.3512	0.2402	0.3408	0.3512	0.1938
H9706	r_s	0.59	0.66	0.42	0.24	0.37	0.03
	p-value	0.0417	0.0205	0.1686	0.4530	0.2353	0.9385
H1178 ^d	r_s	0.32	0.52	0.57	0.60	0.45	0.77
	p-value	0.3146	0.0829	0.0548	0.0384	0.1433	0.0031
Hypeel 696	r_s	0.46	0.53	0.26	0.53	0.54	0.50
	p-value	0.1279	0.0729	0.4182	0.0768	0.0729	0.0999
H3406	r_s	0.49	0.47	0.29	0.49	0.43	0.42
	p-value	0.1243	0.1454	0.3953	0.1243	0.1854	0.1927
TSH28	r_s	0.26	0.32	0.24	0.35	0.33	-0.01
	p-value	0.4183	0.3121	0.4491	0.2635	0.2973	0.9774
TSH18	r_s	0.29	0.26	0.12	0.25	0.27	0.32
	p-value	0.3675	0.4151	0.7142	0.4399	0.4021	0.3065

^a sAUDPC is calculated from defoliation values for each cultivar using their respective DBH defoliation values where Y_i is percent defoliation at day X_i , and Y_{i-1} is percent defoliation at day X_{i-1}

$$\text{AUDPC} = \sum [((Y_i + Y_{i-1}) (X_i - X_{i-1}))/2] \text{ standardized for the length of assessment period becomes:}$$

$$\text{sAUDPC} = \text{AUDPC}/\text{number of days}$$

^b DSI is the relative weight of the number of lesions, calculated as;

$$\text{DSI} = \frac{\sum [(\text{class no.}) (\text{no. of fruit in each class})]}{(\text{total no. fruit per sample}) (\text{no. classes} - 1)} \times 100$$

^c *Rs* values in the first row reflect the relationship between variables summed over all treatments

^d Treatment missing one plot in 2018 fruit trial due to a shortage of transplants.

Table A3.5 Spearman's ranked correlation (r_s) coefficients for foliar standardized area under the disease progress curve (sAUDPC) measured in nine tomato cultivars foliar inoculated with *X. gardneri* seven to ten days after transplanting in foliar resistance field trials, and the incidence of fruit symptom variables from 100 red fruit randomly subsampled from a 2 m section of twin-row in nine tomato cultivars target inoculated with *X. gardneri* at the reproductive clusters in two inoculation timings at the reproductive stage and sorted by fruit trial randomization. Foliar and fruit resistance trials completed at the University of Guelph, Ridgetown ON, 2016-2018.

Cultivar		sAUDPC ^a x DSI ^b	sAUDPC x Any	sAUDPC x <5 mm	sAUDPC x ≥ 5 mm	sAUDPC x Not Split	sAUDPC x Split
All	r_s	0.27 ^c	0.32	0.25	0.34	0.29	0.25
	p-value	0.0047	0.0007	0.0081	0.0003	0.0025	0.0108
CC337	r_s	0.56	0.56	0.47	0.55	0.46	0.35
	p-value	0.0565	0.0604	0.1203	0.0618	0.1312	0.2655
N3306	r_s	0.27	0.25	0.14	0.39	0.23	0.48
	p-value	0.3978	0.4249	0.6661	0.2081	0.4802	0.1111
H5108	r_s	0.15	0.20	0.12	0.28	0.16	0.45
	p-value	0.6503	0.5346	0.7106	0.3849	0.6269	0.1437
H9706	r_s	0.21	0.22	0.06	-0.09	0.08	-0.09
	p-value	0.5098	0.4947	0.8447	0.7908	0.8096	0.7812
H1178 ^d	r_s	0.56	0.52	0.53	0.37	0.42	0.34
	p-value	0.0750	0.0972	0.0911	0.2657	0.1983	0.3094
Hypeel 696	r_s	0.54	0.59	0.55	0.60	0.67	0.18
	p-value	0.0721	0.0431	0.0624	0.0403	0.0178	0.5679
H3406	r_s	0.66	0.67	0.69	0.69	0.67	0.60
	p-value	0.0190	0.0171	0.0127	0.0139	0.0171	0.397
TSH28	r_s	0.38	0.34	0.07	0.50	0.27	0.42
	p-value	0.2256	0.2870	0.8244	0.0941	0.4007	0.1756
TSH18	r_s	-0.21	-0.24	-0.09	-0.23	-0.19	-0.03
	p-value	0.5186	0.4552	0.7740	0.4640	0.5607	0.0926

^a sAUDPC is calculated from defoliation values for each cultivar using their respective DBH defoliation values where Y_i is percent defoliation at day X_i , and Y_{i-1} is percent defoliation at day X_{i-1}

$$\text{AUDPC} = \sum [((Y_i + Y_{i-1}) (X_i - X_{i-1}))/2] \text{ standardized for the length of assessment period becomes:}$$

$$\text{sAUDPC} = \text{AUDPC}/\text{number of days}$$

^b DSI is the relative weight of the number of lesions, calculated as;

$$\text{DSI} = \frac{\sum [(\text{class no.}) (\text{no. of fruit in each class})]}{(\text{total no. fruit per sample}) (\text{no. classes} - 1)} \times 100$$

^c *Rs* values in the first row reflect the relationship between variables summed over all treatments

^d Treatment missing one plot in 2018 fruit trial due to a shortage of transplants.