OCCURRENCE OF BACTERIAL SPOT DISEASE IN ILLINOIS TOMATO FIELDS, CHARACTERIZATION OF THE CASUAL AGENTS, AND MANAGEMENT OF THE DISEASE

BY

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THESIS

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ABSTRACT

Bacterial spot, caused by *Xanthomonas* spp., is one of the most important diseases of tomato in Illinois. Field surveys were conducted in 2017, 2018, and 2019, to assess occurrence of bacterial spot disease in commercial tomato fields. Severity of foliage and fruit infections were recorded, and symptomatic samples for isolation of the pathogen were collected from three-to-five cultivars in three different farms in each of northern, central, and southern regions of Illinois. In 2017 and 2019, average disease severity was the highest in southern Illinois, while it was the highest in northern Illinois in 2018. Incidence of symptomatic fruit was generally low. During the surveys, 266 isolates were collected and identified as *Xanthomonas gardneri* or *X. perforans* using *Xanthomonas*-specific *hrp* primers. Eighty-five percent of *Xanthomonas* isolates identified from northern region were *X. gardneri*, whereas 70% of isolates from southern region were *X. perforans*. Isolates from the central region were 55 and 45% *X. perforans* and *X. gardneri*, respectively. Multilocus sequence analysis using six housekeeping genes (*fusA, gap-1, gltA, gyrB, lepA*, *lacF)* revealed five and four distinct genetic groups for *X. gardneri* and *X. perforans*, respectively.

In addition to *Xanthomonas*, non-*Xanthomonas* bacterial genera were isolated from the diseased fields, with the majority of the isolates being classified as *Microbacterium, Pantoea,* and *Pseudomonas*. Field experiments were conducted in two different locations (Fayette and Champaign Counties), using two different tomato cultivars, 'Red Deuce' and 'Mt. Fresh'. The results showed that plots treated with the combination of copper hydroxide (Kocide-3000 46.1DF) and mancozeb (Manzate PRO Stick) had the lowest disease severity. Field isolates of *X. gardneri* and *X. perforans* were grown on mannitol-glutamate-yeast agar (MGYA) amended with 0.8 mM copper sulfate. Two copper resistance genes, *copA* and *copM*, were present only in the isolates that developed colonies on the copper-amended MGYA. Additionally, *in vitro* laboratory studies were conducted to determine the effectiveness of selected chemical compounds and biopesticides at

preventing colony formation of copper resistant and copper sensitive isolates of *X. gardneri* and *X. perforans*. The results showed that the combination of copper hydroxide (Kocide-3000 46.1DF) and mancozeb (Manzate PRO-stick) produced the largest inhibition zone for the copper-sensitive isolates of both species (*X. perforans* and *X. gardneri*). In contrast, the combination of *Bacillus amyloliquefaciens* strain D747 (Double Nickel LC) and copper octanoate (Cueva) produced the largest inhibition zone for copper resistant isolates of both species.

To my family and friends

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CHAPTER 1: INTRODUCTION AND OVERVIEW

Tomato. Tomato, *Solanum lycopersicum*, is a member of the Solanaceae family, and one of the most popular and widely grown crops throughout the world (Knapp and Peralta, 2016). The family Solanaceae consists of more than 3,000 species, and some of the economically important species in this family include *S. tuberosum* (potato), *S. melongena* (eggplant), *Datura stramonium* (datura), and *Petunia* spp. (Petunia) (Knapp, 2002).

The origins of domesticated tomato are debatable, since it is considered to have been spread globally for breeding purposes (Grandillo et al., 2011). Various studies have suggested the introduction of domesticated tomato plants from Peru, citing both historical and botanical reasons (Luckwill, 1943; Müller, 1940), whereas Jenkins (1948) suggested that tomato was introduced to Europe from Mexico. Blanca et al. (2012) concluded pre-domestication of tomato occurred in the Andean region in South America.

Tomato production. The United States (US) is the third largest tomato producing country in the world (FAOSTAT, 2017). In 2017, the total harvested area for tomato production in the US was 126,070 hectares (ha) yielding 10,910,990 tons. Both fresh-market and processing tomatoes are grown in the US. In 2015, the total area for fresh-market tomato production was 1,477,000 ha while the total area for processing tomato production was 826,244 ha (USDA, 2016). Florida and California are the top states for tomato production, followed by Tennessee and North Carolina. In the Midwest, Ohio and Michigan are the top tomato producing states (Statista, 2015). Approximately 1,000 ha of fresh-market tomato are grown annually in Illinois (Babadoost, personal communication).

Tomato diseases in Illinois. The major tomato diseases in Illinois include fungal diseases (Alternaria blight, anthracnose, Sclerotinia blight, Septoria leaf blight), viral diseases (cucumber

mosaic, tobacco mosaic, tomato mosaic), and bacterial diseases (bacterial canker, bacterial speck, bacterial spot), as well as several abiotic disorders (Babadoost and Yu, 2017).

Alternaria blight, also known as early blight, is caused by the fungus *Alternaria solani* and is considered one of the most important fungal disease of tomatoes. Early blight symptoms occur on all above-ground parts of tomato plants and during all stages of plant development. The disease appears as small brownish brown lesions on older leaves that can enlarge up to 6 mm in diameter. Spots can be distinguished by concentric rings with a dark brownish color. *A. solani* is known to overwinter in infected plant debris as well as in soil, seeds, volunteer tomato plants, and other solanaceous hosts. This disease is severe during mild (24-29˚C) and rainy conditions (Jones, 1991).

Anthracnose, caused by the fungus *Colletotrichum coccodes,* is a common fruit disease of tomato. It is primarily a disease of ripe and over-ripe tomato fruit. Symptoms first become visible on ripe fruit as small, circular, indented spots in the skin. A lesion may enlarge up to 12 mm in diameter and sink into the fruit. As the spots expand, they develop dark centers or concentric rings of dark specks, which are the spore-producing bodies (acervuli) of the fungus. The pathogen survives annually as microsclerotia, often in association with plant debris. Under wet and warm conditions, the microsclerotia germinate and produce hyphae as well as conidia within the acervuli. Microsclerotia can survive for long periods in the soil under adverse environmental conditions, while plant infection occurs when temperatures are between 10 and 30°C and the air is humid.

Sclerotinia blight, also known as white mold, is caused by the fungus *Sclerotinia sclerotiorum*. Sclerotinia blight is usually noted during the flowering when water-soaked areas can develop at leaf axils and stem joints. The disease then expands to the stems, causing the light gray appearance of cottony mycelium on infected stems, which is a result of the production the sclerotia, though sclerotia can also be produced inside the stems. The pathogen overwinters as sclerotia in the soil, and in spring it can germinate to produce cup-shaped white or yellow apothecia that contains the

cylindrical asci. Asci produce the ascospores that are ejected in the air and carried by wind onto host plants. Low-laying areas usually have the highest disease intensity, while poor air circulation and moisture retention likely aggravates disease development. Cooler temperatures (11-15˚C) favor the development of apothecia (Pohronezny, 1991).

Septoria leaf blight, caused by the fungus *Septoria lycopersici,* is a devastating disease of tomato in Illinois (Babadoost and Yu, 2017). This disease appears as small, water-soaked lesions on leaf petioles, stems, blossoms, and flower stalks, which can enlarge to 3-6 mm in diameter. As the lesions mature, they develop a dark margin with a grayish center. Pycnidia, which contains the conidia, develop in the lesion and spores are released from the pycnidia when rain splashed onto the lesions. The pathogen survives in infected plant debris present on the soil surface. The disease is severe during the prolonged periods of warm, wet weather conditions (Stevenson, 1991).

Several diseases caused by viruses can affect tomato production. Cucumber mosaic, caused by *Cucumber mosaic virus* (CMV), occurs worldwide and can affect more than 800 plant species present in at least 100 families. CMV caused mosaic disease symptoms on leaves. CMV symptoms in early stages are yellow, bushy, and considerably stunted. Two related viruses include Tobacco mosaic, caused by *Tobacco mosaic virus* (TMV), and tomato mosaic, caused by *Tomato mosaic virus* (ToMV). Symptoms caused by TMV and ToMV are generally similar in appearance, and symptoms include a chlorotic mosaic patterning and developmental distortion of leaves.

Bacterial canker of tomato, caused by *Clavibacter michiganensis* subsp. *michiganensis (Cmm)*, is another important disease of tomato in Illinois. *Cmm* survives on plant debris in soil, weed hosts, volunteer plants, and infected seeds. The bacterium is disseminated by splashing water and by mechanical means (e.g., pruning tools). Wilting is the major symptom of this disease; early symptom include downward turning of lower leaves, marginal necrosis of leaflets, and leaf wilting. Infected stems exhibit light yellow-to-brown streaks in the vascular tissues, which later turn

reddish brown and are most prominent around the leaf nodes. Lesions on fruits are generally referred as “bird’s eye” spots, with raised lesions that include brown centers surrounded by a white halo (Gitaitis, 1991).

Bacterial speck, caused by *Pseudomonas syringae* pv. *tomato*, is a damaging disease of tomato plants in Illinois (Babadoost and Yu, 2017). Symptoms of this disease appear as dark brown-to-black lesions with light green halos occurring on leaflets, stems, petioles, peduncles, pedicles, and sepals. Spots develop on fruit as small lesions or specks, and can become enlarged as the fruits grow in size (Jones, 1991).

Bacterial spot disease. Bacterial spot, caused by *Xanthomonas* spp., is the most important bacterial disease of tomato plants in Illinois (Babadoost and Yu, 2017). Bacterial spot disease can cause more than 50% yield loss. The disease is more prevalent in warm and humid areas. *Xanthomonas* bacteria can survive on infected plant debris and volunteer plants. The development of symptoms is generally favored by moist environmental conditions and temperatures of 24-30°C. The bacteria are disseminated by the wind-driven rain and penetrate aerial plant tissues through wounds and open stomata. *Xanthomonas* spp. can infect all aboveground plant parts to cause brown, circular spots on leaves, stems, and fruits (Jones, 1991).

Pathogen causing bacterial spot disease. According to Doidge (1921), bacterial spot of tomato was first observed in South Africa and the disease was originally thought to be caused by one group of strains called *Xanthomonas campestris* pv. *vesicatoria* (Stall et al., 1994; Vauterin et al., 1995). However, more recent studies showed that the causal agents could be divided in two strain groups, A and B, which were identified as *X. axonopodis* pv. *vesicatoria* (strain A) and *X. vesicatoria* (strain B) (Vauterin et al., 1995). In 1953, Sutic isolated a bacterium from tomato in the former Yugoslavia and named it *Pseudomonas gardneri*, although it was later renamed as *X. vesicatoria* (Dye, 1966). However, it was later characterized as *X. gardneri* (group D) based on DNA-DNA

hybridization, and determined to be a species distinct from the other two groups (Deley, 1978). Jones et al., (1995) isolated a fourth genetically distinct group from tomato in Florida, which was classified as a new race of *X. campestris* pv. *vesicatoria*, as group C. In 2004, Jones and colleagues (Jones et al., 2004) determined that groups C and D were distinct *Xanthomonas* species. Currently, the following pathogens are considered the casual agents of bacterial spots disease of tomato: *X. euvesicatoria* (group A), *X. vesicatoria* (group B), *X. perforans* (group C), and *X. gardneri* (group D) (Jones et al., 2000; Jones et al., 2004).

Symptoms of bacterial spot. Bacterial spot of tomato is characterized by necrotic lesions on leaves, stems, flowers, and fruits (Jones et al., 1991). During its initial stages, symptoms develop as circular water-soaked lesions that dry out to give a greasy appearance, and eventually turn dark-brown to black (Vallad et al., 2004). Lesions on the leaves caused by *X. perforans* often have a shot-hole appearance when conditions favor rapid bacterial multiplication, whereas *X. gardneri* has a water-soaked appearance (Stall et al., 2009). *X. euvesicatoria* and *X. vesicatoria* have been generally associated with fruit lesions, although recent publications have shown *X. gardneri* association with deep, large fruit lesions (Ma et al., 2011; Miller, 2012).

Epidemiology of bacterial spot. Together, high temperatures, high relative humidity, high plant density, and overhead irrigation create an ideal environment for the development and spread of bacterial spot disease of tomato (Potnis et al., 2015). However, small differences in preferred temperature and precipitation conditions exist between the species (Araujo et al., 2010). Disease caused by *X. gardneri* is more prevalent in cooler temperature conditions (Jones et al., 1988), and it can cause more severe disease at 20°C compared to the other three species (Araujo et al., 2010). Generally, *Xanthomonas* spp. are disseminated within a field by environmental conditions such as wind-driven rain, and by mechanical means such as grafting, clipping, tying, harvesting, and spraying pesticides (Lindeman and Upper, 1985; McInnes et al., 1988). Additionally, it has been

reported that *Xanthomonas* spp. from tomato can also survive epiphytically (McGuire et al., 1991), which may aid in bacterial dispersal.

Genetic variation in bacteria. Genetic variation in phytopathogenic bacteria has been detected using phenotypic and genotypic tests. Alterations of existing DNA and exchange of new genetic material are important mechanisms for bacterial evolution. Types of DNA changes include mutations, deletions, insertions, inversions, translocations, and duplications (Bryant et al., 2012). Point mutations in bacterial DNA occur non-randomly, and depending on the site of the mutation, can have important functional consequences. DNA sequence changes and genomic structural variations can both lead to bacterial evolution (Bryant et al., 2012). This accumulation of genomic alterations can be used to define phylogenetic relationships between bacterial species (Wicker et al., 2012).

The exchange of genetic material between different bacteria can occur via three mechanisms: transformation, conjugation, and transduction. Transformation occurs when DNA is transferred from a donor bacterium and incorporated into the genome of a recipient bacterium. Conjugation is the process whereby the bacteria acquires new genetic material through the pilus structure. Transduction is the process by which a bacteriophage transfers genes from one bacterium to another. (Andrew and Harris, 2000; Robertson and Meyer, 1992; Synder and Champness, 2007).

Several different systems such as carbon source utilization, panels of biochemical reactions, cellular fatty acid profiles (Holmes et al., 1994; Miller and Rhoden, 1991; Osterhout et al., 1991; Von Graaeventiz et al., 1991) are used to identify bacteria. These systems, however, depend on subjective judgement for interpretation of their results (Stager and Davis, 1992) and are generally complemented by genotypic tests. The 16S ribosomal RNA (rRNA) gene is present in almost all bacteria, and has been used for identification of bacterial isolates (Janda and Abbott, 2007; Lane, 1991; Patel, 2001). The function of the 16S rRNA gene has not changed over time (Patel 2001),

and its high sequence conservation allows for accurate genus-level identification, though it is less reliable for species identification (Mignard and Flandrois, 2006; Drancourt et al., 2000). Additionally, 16S rRNA gene sequencing is easier to perform than DNA-DNA hybridization analysis (Janda and Abbott, 2007).

Molecular identification of *Xanthomonas* spp. For the past few decades, researchers identified *Xanthomonas* spp. using diagnostic methods based on the *hrp* gene clusters. *Hrp* gene clusters are highly conserved among a number of phytopathogenic bacteria (Fenselau et al., 1992; Hwang et al., 1992), including *Pseudomonas solanacearum* (Boucher et al., 1987), *Erwinia amylovora* (Beer et al., 1991), and *X. campestris* pv. *euvesicotaria* (Bonas et al., 1991). These genes are usually required for the production of symptoms on susceptible hosts, and may cause a hypersensitive reaction on resistant or nonhost plants (Willis et al., 1990).

Leite et al. (1994) was the first to develop a diagnostic tool using the *hrp* gene cluster to identify bacterial spot strains. This PCR-based assay targeted different *hrp* genes and used a restriction endonuclease analysis (REA) to differentiate between 28 *X. campestris* pathovars. Based on this work, Obradovic et al. (2004) developed PCR primers to amplify a 420 base pair (bp) fragments of *hrp*B7 from the four bacterial spot species that infect tomato. In a further technological development, Strayer et al. (2016) developed a method to simultaneously detect all four bacterial spot species using multiplex quantitative PCR targeting the *hrp*B7 gene. Besides the methods utilizing *hrp*B7, other protocols were developed, including the one by Koenraadt et al. (2009) that identified specific PCR primers based on unique binding patterns from AFLP (Amplified Fragment Length Polymorphism) analysis. Additionally, Araujo et al. (2012) extracted DNA from 30 *X. euvesicatoria*, 30 *X. vesicatoria*, 50 *X. gardneri*, and 50 *X. perforans* isolates, and developed a multiplex PCR assay to differentiate between the four bacterial species causing bacterial spot of

tomato. Amplification of *hrp*B7 genes and multiplex PCR assay are still used as routine diagnostic today.

Genetic variation in tomato *Xanthomonas* spp. Genotypic characterization of prokaryotes can be performed using multilocus sequence typing (MLST; also known as multilocus sequence analysis, or MLSA), which differentiates bacterial strains using a small number of allelic differences in housekeeping genes (Maiden et al., 1998). Six housekeeping genes (*fusA, gap-1, gtlA, gyrB, lacF,* and *lepA)* were used to create a MLSA database of *Xanthomonas* strains (Almeida et al., 2010), where degenerate primers for housekeeping genes were developed based on allelic differences between *X. campestris* ATCC 33913 (da Silva et al., 2002), *X. axonopodis* subsp. *citri* strain 306 (da Silva et al., 2002), and *X. oryzae* pv. *oryzae* KACC10331 (Lee et al., 2005). Later, Timilsina et al. (2015) performed MLSA using these six housekeeping genes to characterize the shift in species composition and genetic diversity for the four *Xanthomonas* spp. known to cause bacterial spot disease in tomato and pepper plants. Their results indicated that homologous recombination has occurred throughout the genomes of these four species.

Copper resistance in *Xanthomonas.* Due to its low cost and minimal toxicity to humans, copper compounds have been popular for management of bacterial diseases in agricultural systems (Areas et al., 2018; Marco and Stall, 1983). However, excessive use of copper compounds has led to development of copper-resistant strains of *Xanthomonas campestris* pv. *vesicatoria*, which were first reported in Florida in the early 1980s (Marco and Stall, 1983), and later reported in pepper and tomato fields in both Oklahoma and North Carolina (Bender et al., 1990; Ritchie and Dittapongpitch, 1991). Worldwide, copper resistant strains of *X. campestris* pv. *vesicatoria* on pepper, *X. perforans* on tomato, and *X. euvesicatoria* on pepper have been reported in Australia, Canada, and Brazil, respectively (Abbasi et al., 2015; Areas et al., 2018; Martin, et al., 2004).

The effectiveness of copper compounds is dependent on the concentration of free copper ions; however, when the ions are chelated by organic compounds such as those found in most crop production fields, they lose their toxicity to the bacteria (Menkissoglu and Lindow, 1991; Zevenhuizen et al., 1979). It has been reported that the effectiveness of copper compounds increased when used in combination with ethylene bisdithiocarbamate fungicides, such as mancozeb, to manage the phytopathogens *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* (Marco and Stall, 1983; Parsons and Edgington, 1981). Similarly, Lee et al. (1993) reported increased control of *Xanthomonas campestris* pv. *juglandis* when iron was added to fixed copper compounds like Kocide 101 (77% copper hydroxide).

The copper resistance phenotypes for two *Xanthomonas campestris* pv. *vesicatoria* strains was discovered to be encoded by the self-transmissible plasmids pXvCu (Stall et al., 1986) and pXV10A (Bender et al., 1990). The two plasmids were genetically distinct (Bender et al., 1990). Basim et al. (2005) characterized the *Xanthomonas campestris* pv. *vesicatoria* strain XvP26, originally isolated from pepper plants in Taiwan, as containing a 7,652 bp region, which was identified as the copper resistance locus. De La Iglesia et al. (2010) designed the first PCR primers to detect bacterial copper resistance genes, specifically through amplification the copper P-type ATPases genes. Behlau et al. (2011) identified eight opening reading frames (ORFs) in the copper operon of *X. citri* subsp. *citri* A44 , including genes *copL, copA, copB, copM, copG, copC, copD,* and *copF*. Except for *copC* and *copD*, all other ORFs were also present in the copper-resistant strain *X. alfalfa* subsp. *citrumelonis* 1381 (Behlau et al., 2011).

Biopesticides for bacterial diseases. The development of biopesticides for management of plant bacterial diseases has been intensively studied over the past 50 years (Wilson, 1997; Wilson et al., 1998). Some biopesticides have been used commercially for the management of bacterial diseases such as fire blight of pear, caused by *Erwinia amylovora* (Wilson, 1998; Wilson and

Lindow, 1993), and crown gall disease, caused by *Agrobacterium tumefaciens* (Clare, 1993). There have been numerous studies performed under laboratory and greenhouse conditions for management of *Xanthomonas* species (Assis et al., 1996; Bora et al., 1993; Hsieh and Buddenhagen, 1974; Jindal and Thind, 1993; Stromberg et al., 2000; Verma and Singh, 1987; Zhang et al., 1997).

There have been several studies that reported reduction of disease symptoms using biological agents, for management of bacterial spot of tomato. Two studies found that bacteriophages could significantly decrease disease caused by *Xanthomonas* spp. on tomato (Balogh et al., 2003; Obradovic et al., 2004). Byrne et al. (2005) reported that the bacteria *Pseudomonas syringae* Cit7 and *P. putida* provided the greatest reduction in disease severity on foliage caused by bacterial spot of tomato (Wilson et al., 2002). Additionally, the bacteria *Rahnella aquatilis* was found to have antagonistic properties against *X. campestris* pv. *vesicatoria* when applied through a foliar spray (El-Hendawy et al., 2005), while treatment of tomato seeds with a *Bacillus* sp. isolate reduced the severity of bacterial spot diseased caused by *X. gardneri* by 48% (Naue et al, 2014). Similarly, other studies have identified some saprophytic fungi such as *Memmoniella levispora, Periconia hispidula, Zygosporium echinosporum,* and *Chloridium virescens* var. *virescens* as potential biocontrol agents for management of bacterial spot disease of tomato (Peitl et al., 2017).

Recently, a few studies tested mutant strains of phytopathogenic bacteria for use as biopesticides (Hanemian et al., 2013). The 75-3S *hrpG* mutant of *X. campestris* pv. *vesicatoria* provided significant management of bacterial spot of tomato under both greenhouse and field conditions, with reported efficacy that was significantly higher than other *hrp* mutants (75-3 *hrpX*, *hrpF*, or *hrpE1*), two biological agents (*P. syringae* Cit7 and *P. putida* B56), and copper/ ethylene bisdithiocarbamate combinations (Moss et al., 2007). Hert et al. (2009) reported a significant reduction of a *X. euvesicatoria* population with the use of attenuated mutant of *X. perforans*(91-

118Δ*opgH*Δ*bcnB*) with deletion of osmoregulated periplasmic glucan gene (*opgH*) and lacking bacteriocin activity (*bcnB*) when compared to standard management treatments such as the combination of copper hydroxide and mancozeb.

THESIS OBJECTIVES

The overall goal of this research was to identify bacterial species associated with bacterial spot disease of tomato in Illinois and to evaluate the effectiveness chemical management of the disease. The specific objectives of the study were to:

1. Survey commercial tomato fields in Illinois to determine the incidence and severity of bacterial spot disease found on tomato leaves and fruit;
2. Identify the bacterial species causing bacterial spot disease of tomato;
3. Determine the genetic variation among pathogenic isolates causing bacterial spot disease of tomato;
4. Identify the non-pathogenic bacteria associated with bacterial spot symptoms on tomato; and
5. Determine the efficacy of chemical compounds and biopesticides for management of bacterial spot disease of tomato in Illinois.

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CHAPTER 2: OCCURRENCE OF BACTERIAL SPOT DISEASE IN ILLINOIS TOMATO FIELDS AND CHARACTERIZATION OF THE CASUAL AGENTS XANTHOMONAS PERFORANS AND X. GARDNERI

ABSTRACT

Bacterial spot, caused by *Xanthomonas* spp., is one of the most important diseases of tomato in Illinois. Field surveys were conducted in 2017, 2018, and 2019 to assess occurrence of bacterial spot in commercial tomato fields. Severity of foliage and fruit infection was recorded, and symptomatic samples were collected from three to five cultivars in three different farms in each of northern, central, and southern regions of Illinois. In 2017 and 2019, average disease severity was the highest in southern Illinois, while it was the highest in northern Illinois in 2018. Incidence of symptomatic fruit was generally low. Over three years, 266 isolates were identified as *Xanthomonas gardneri* and *X. perforans* using *Xanthomonas*-specific *hrp* primers. Eighty-five percent of *Xanthomonas* isolates identified from northern region were *X. gardneri*, whereas 70% of isolates from southern region were *X. perforans*. Isolates from the central region were identified as 55% and 45% for *X. perforans* and *X. gardneri*, respectively. Multilocus sequence analysis using six housekeeping genes (*fusA, gap-1, gltA, gyrB, lepA*, *lacF)* revealed five and four different genetic groups for *X. gardneri* and *X. perforans*, respectively. In addition to *Xanthomonas*, nine non-*Xanthomonas* bacterial genera were isolated from the samples, with the majority of isolates being classified as *Microbacterium, Pantoea,* and *Pseudomonas*.

INTRODUCTION

Bacterial spot, caused by *Xanthomonas* spp., is the most important bacterial disease affecting tomato production in Illinois. Bacterial spot was first identified in South Africa (Doidge, 1921). Originally, bacterial spot was thought to be caused by only one species, *Xanthomonas campestris* pv. *vesicatoria* (Stall et al., 1994; Vauterin et al., 1995); however, subsequent studies divided it into two species: *X. axonopodis* pv. *vesicatoria* (group A) and *X. vesicatoria* (group B) (Vauterin et al., 1995). Currently, the following species are considered to be the casual agents of the bacterial spot disease complex of tomato: *X. euvesicatoria* (group A), *X. vesicatoria* (group B), *X. perforans* (group C), and *X. gardneri* (group D) (Jones et al., 2000; Jones et al., 2004).

Bacterial spot of tomato is characterized by necrotic lesions on leaves, stems, flowers, and fruits (Jones et al., 1991). During the initial stages of disease development, symptoms develop as circular water-soaked lesions that dry out to give a greasy appearance, and eventually turn dark brown-to-black (Vallad et al., 2004). Some species-specific symptoms include shot-hole type lesions caused by *X. perforans* and lesions with a more water-soaked appearance caused by *X. gardneri* (Stall et al., 2009). *X. euvesicatoria* and *X. vesicatoria* have been generally associated with lesion development on fruits, though recent publications have also shown *X. gardneri* causing deep, large fruit lesions (Ma et al., 2011; Miller, 2012). Generally, the disease favors warmer and more humid conditions for progression and spread (Araujo et al., 2010); however, *X. gardneri* appears more often in cooler temperatures (Jones et al., 1988) and causes more severe disease at 20°C than the other three species (Araujo et al., 2010). *Xanthomonas* bacteria are disseminated within a field by wind-driven rain and by mechanical means such as grafting, clipping, tying, harvesting, and spraying pesticides (Lindeman and Upper, 1985; McInnes et al., 1988).

During the past several decades, researchers have utilized diagnostic methods based on the *hrp* gene clusters which are highly conserved among a number of phytopathogenic bacteria (Fenselau

et al., 1992; Hwang et al., 1992). Leite et al. (1994) was the first to use *hrp* gene clusters as a diagnostic tool for identification of bacterial spot species and pathovars. Fragments of different *hrp* genes were amplified using a polymerase chain reaction (PCR) assay, followed by restriction digestion using endonucleases to allow detection of 28 different *X. campestris* pathovars. Based on this work, Obradovic et al. (2004) developed specific PCR primers that amplify a 420 base pair (bp) fragment of *hrpB7* from four bacterial spot pathogens causing disease in tomato; this diagnostic method is still used in routine diagnostic tests today.

Genotypic characterization of prokaryotes can be performed using multilocus sequence analysis (MLSA), which differentiates between bacterial strains using a small number of allelic mismatches found in housekeeping genes (Maiden et al., 1998). Six housekeeping genes (*fusA, gap-1, gtlA, gyrB, lacF, lepA)* were used to create a MLSA database of *Xanthomonas* strains (Almeida et al., 2010).

The objectives of this study were to: (i) assess the incidence and severity of bacterial spot disease of tomato in Illinois, (ii) identify the *Xanthomonas* spp. causing the bacterial spot disease, (iii) evaluate the genetic diversity of *Xanthomonas* isolates and examine the phylogenetic relationships using housekeeping genes, and (iv) classify the non-*Xanthomonas* bacteria associated with bacterial spot caused by *Xanthomonas* spp.

MATERIALS AND METHODS

Field surveys. During 2017, 2018, and 2019, field surveys were conducted to assess the severity of bacterial spot disease on tomato cultivars grown in Illinois. Each year, commercially grown tomato farms located in the southern, central, and northern regions of Illinois (Figure 2.1) were visited three or four times throughout the growing seasons (Table 2.1). A total of 13 different cultivars were evaluated, including Biltmore, Brandywine, Carolina Gold, Chef's Choice, Dixie

Red, Heirloom, Jolene, Phoenix, Primo Red, Pony Express Plum, Red Deuce, Red Morning, and Rocky Top.

At each farm, 10 randomly-selected plants from each available cultivar were evaluated for severity of bacterial spot on leaves, stems, and fruits. The scale (0-11) modified from Horsfall and Barratt (1945) was used for the evaluation of severity of the disease. Symptomatic samples were collected for isolation of bacteria associated with the affected tissues (Table 2.1). Samples were stored at 4°C until bacterial isolation was completed within 72 hours post collection.

Isolation and maintenance of bacterial isolates. Bacteria were isolated from collected samples using the procedure reported by Schaad et al. (2001). Plant tissues were washed with tap water to remove soil and other particles, 5 x 5 mm tissue sections containing lesions were cut, and sections were surface-disinfested using 99% ethanol for 60 s. The sections were then washed three times (1 min each time) with sterile distilled water (SDW). Each section was inserted into a 15 ml tube containing 10 ml (SDW) and shaken by hand for 20 s to prepare a bacterial suspension (Schaad et al., 2001). Then, 100 μl of the suspension was transferred onto nutrient agar (NA) and yeast dextrose calcium agar (YDC) media in Petri plates. The plates were incubated at 28°C for 72 hr, at which time well-developed colonies were sub-cultured onto NA media. Isolates were stored in 4 mM sucrose and 20% v/v glycerol at -80°C.

Identification of bacterial isolates. Isolated bacterial colonies were grown on YDC and NA media, and then grouped based on their colony morphology and color. Consistent with Schaad et al. (2001), the colonies with yellow mucoid characteristics on YDC, which were suspected to be *Xanthomonas* spp., were subjected to PCR using RST 65/69 (*hrpB7*) primers, while colonies colored orange, white, pink, etc., were subjected to PCR using primers for 16S rRNA.

Yellow colonies growing on YDC were evaluated for the presence of *hrp*B7 using PCR analysis. Isolates were identified based on the PCR results and amplicon sequencing. First, isolates

were streaked onto NA and incubated at 28℃ for 72 hr. The *Xanthomonas*-specific primers RST 65 (5'-GTCGTCGTTACGGCAAGGTGGTCG-3') and RST 69 (5'-TCGCCCAGCGTCATCAGGCCATC-3'), which amplify a 420 bp fragment of *hrp*B7, (Obradovic et al., 2004) were used for PCR amplification. A 25 μl reaction was set up using Taq DNA polymerase following the manufacturer's recommendation (Omega Bio-Tek Inc., Norcross, GA), and using a small amount of bacteria as the template (Leite et al., 1994). PCR amplification was performed using a ProFlex thermal cycler (Thermo Fisher Scientific, Waltham, MA), with initial denaturation at 95℃ for 5 min; 35 cycles of denaturing at 95℃ for 30 s, annealing at 63℃ for 30 s, and extension at 72℃ for 45 s; followed by a final extension at 72℃ for 5 min (Obradovic et al., 2004). Then, 5 µl of each PCR reaction was analyzed using gel electrophoresis with a 1% agarose gel containing EZ-vision DNA dye (VWR life sciences, OH), run at 100 V for 30 min, and visualized at 470 nm by the Azure Biosystems c400 imager (Dublin, CA).

The remaining PCR product was purified according to manufacturer's recommendation using EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA) and sent for Sanger sequencing at the DNA Services Lab, Roy J. Carver Biotechnology Center, University of Illinois. Sequences were analyzed using the BLASTN database from the National Center for Biotechnology Information (NCBI).

Pathogenicity tests. After the identification of *Xanthomonas* isolates, pathogenicity tests were conducted in a greenhouse on four different tomato cultivars (Brandywine, Dixie Red, Primo Red, and Red Deuce) using a subset of representative isolates from each of the southern, central, and northern regions. Each bacterial culture was grown on NA for 48 hr, colonies were washed by SDW, and the suspension was adjusted to 5 x 10^8 CFU/ml (OD_{600} = 0.3) according to the method reported by Kyeon et al. (2016). Eight- to ten-week-old plants were inoculated by infiltrating 0.5 ml of the bacterial suspension into each leaflet using a needleless syringe. Control plants were

infiltrated with SDW. Development of the symptoms was monitored and recorded 3, 5, and 7 days post inoculation.

Phylogenetic analysis. All the field isolates identified as *Xanthomonas* were used for the development of a phylogenetic tree using MLSA. The isolates were evaluated using the six housekeeping genes *fusA, gap-1, gltA, gyrB, lacF,* and *lepA* (Almeida et al., 2010). Gene sequence fragments were obtained via Sanger sequencing, and compared to the corresponding sequences obtained from the following reference sequences, with NCBI accession or WGS information listed in parentheses: *X. gardneri* JS749-3 (NZ_CP018728); *X. gardneri* ICMP7383 (NZ_CP018731.1); *X. perforans* 91-118 (NZ_CP019725; representative Florida group 1, Xp-FL1); *X. perforans* Xp3-15 (JZVG01; representative Florida group 2, Xp-FL2); *X. perforans* Xp4-20 (JZUZ01; representative Florida group 3, Xp-FL3); and *X. euvesicatoria* 85-10 (NC_007508.1). *X. cucurbitae* (NZ_MDED00000000.1) was used as outgroup.

All the gene sequence fragments, either obtained via Sanger sequencing or extracted from whole genome sequences, were aligned using CLUSTALW within MEGA 10.0.5 (Kumar et al., 2018) and trimmed to the same length using the same starting position. For each isolate, the trimmed sequences for the six genes were concatenated for a total of 3,092 nucleotides. All concatenated sequences were aligned, and the Akaike Information Criterion (AIC) within jModeltest 1.1 (Posada and Buckley, 2004) was used to select the nucleotide substitution model that best fit the aligned sequences. The Tamura 3-parameter model with gamma distribution and with invariant sites (T92 + G + I) was utilized for constructing the phylogenetic trees. The maximum likelihood tree was determined using the concatenated sequences with 1,000 bootstrap samples. Genetic groups were defined as isolates having less than 10 nucleotide differences.

Identification of non-*Xanthomonas* isolates. All of the isolates that tested negative for *hrp*B7 amplification were further analyzed for genus identification and determination of pathogenicity.

Identification of non-*Xanthomonas* isolates was achieved by using 16S rRNA sequencing. PCR amplification of 16S rRNA was performed using the primer set 27 F (5'-AGAGTTTGATCMGGCTCAG-3') and 1492 R (5'- GGTTACCTTGTTACGACTT- 3') (Lane, 1991). The PCR amplification, purification, and sequencing was performed as described above except for the PCR amplification conditions, which consisted of 35 cycles of denaturation at 95°C for 45 s, annealing at 55°C for 30 s, and extension at 72°C for 90 s. Sequences were analyzed using the BLASTN database from the National Center for Biotechnology Information (NCBI) in order to assign each isolate to a particular genus. Isolates that did not have a match in BLASTN database were labeled as unknown.

Pathogenicity of non-*Xanthomonas* isolates were conducted in greenhouse similar to methods described above in two different cultivars (Red Deuce and Brandywine).

RESULTS

Incidence and severity of bacterial spot. Bacterial spot was observed in 26 of 27 fields visited during 2017-2019. In the 2017 and 2019 growing seasons, the average disease severity was highest in southern Illinois, while in 2018 it was the highest in northern Illinois (Table 2.2). The greatest range of disease severity (0 to 88%) was observed in northern Illinois in 2019, and the lowest range was observed in central Illinois in 2017 (3 to 4.5%). Overall, disease severity was highest during the 2019 growing season (Table 2.2). Incidence of symptomatic fruit was generally low, with the overall highest incidence occurring in 2019 and the lowest in 2017 (Table 2.3).

Identification of *Xanthomonas* spp. A total of 266 isolates that produced the 420 bp *hrp*B7 amplicon by PCR analysis were identified as *Xanthomonas* spp. Of the 266 isolates, 221 were from foliar tissues and 45 from fruits (Table 2.4). Of the 139 isolates from the southern region, 101 and 38 isolates were identified as *X. perforans* and *X. gardneri*, respectively. In contrast, of the 91 isolates from the northern region, 13 and 78 isolates were *X. perforans* and *X. gardneri*,

respectively. Among the 36 isolates from the central region, 19 and 17 isolates were *X. perforans* and *X. gardneri*, respectively (Table 2.4).

Pathogenicity test. Representative isolates were tested for pathogenicity as determined by the development of lesions on inoculated leaves 3 days post inoculation. All the isolates tested caused disease symptoms in tomato leaves. Bacteria were re-isolated from the infiltrated leaves and tested using PCR for the production of the *hrp*B7 gene fragment. Plants infiltrated with SDW did not develop any lesions and no bacteria were isolated from tissues infiltrated with SDW.

Multilocus sequence analysis of isolated *Xanthomonas* spp. Isolates with fewer than 10 nucleotide changes out of 3,092 total base pairs were grouped together, resulting in five groups for *X. gardneri* (Xg-IL1, Xg-IL2, Xg-IL3, Xg-IL4, and Xg-IL5) and four groups for *X. perforans* (Xp-IL1, Xp-IL2, Xp-IL3, and Xp-IL4). Phylogenetic analysis was performed using a representative isolate from each group, along with reference isolate. *X. gardneri* Xg-IL1 was the most prevalent (119 isolates) (Table 2.5), and these isolates were most similar to the reference isolate *X. gardneri* JS749-3 (Richard et al., 2017). Xg-IL1 isolates were found in all three regions and from multiple farms within each region. In contrast, groups Xg-IL2, Xg-IL3, Xg-IL4, and Xg-IL5 were isolated from individual farms in the northern region. Likewise, *X. perforans* Xp-IL1 was the most prevalent (90 isolates) (Table 2.5), and was most similar to the reference strain *X. perforans* 91-118 (Figure 2.2). Xp-IL1 isolates was collected from multiple farms in the central and southern regions. For Xp-IL2, 36 isolates were from a single farm in the southern region and was found to be similar to Florida group 2 isolates (Xp-FL2; Figure 2.2). Xp-IL3 and Xp-IL4 strains were isolated from single farms in the southern and northern regions, respectively.

Identification of non-*Xanthomonas* bacteria. A total of 714 bacterial isolates were collected from symptomatic tomato tissues that neither developed characteristic yellow colonies of *Xanthomonas* on YDC nor produced the 420 bp product using RST 65/69 primers. These non-

Xanthomonas isolates mostly belonged into nine bacterial genera, including *Agrobacterium, Bacillus, Curtobacterium, Microbacterium, Paenibacillus, Pantoea, Psuedomonas, Rhizobium,* and *Stenotrophomonas.* The majority of isolates were identified as *Microbacterium, Pantoea*, and *Pseudomonas* spp. (Table 2.6). To determine whether these bacteria could cause infection on tomato plants, representative isolates were syringe-infiltrated into leaves of Red Deuce and Brandywine plants grown in a greenhouse. None of the leaves developed lesions 7 days post infiltration, suggesting that only the *Xanthomonas* isolates were responsible for causing the disease symptoms observed in the production fields.

DISCUSSION

This is the first field survey for identification of the bacterial spot pathogens infecting tomato foliage and fruit in Illinois. We utilized field surveys to assess the incidence and severity of disease, and combined conventional and molecular analysis for genotyping with a multilocus sequencing approach for evaluating the genetic diversity of *Xanthomonas* spp. causing bacterial spot disease in Illinois. None of the cultivar grown in commercial fields were found to be resistant to bacterial spot. Disease severity on foliar tissues was higher in southern Illinois, while disease incidence on fruits was higher in northern Illinois.

X. gardneri and *X. perforans* were identified as the causal agents of bacterial spot in Illinois. *X. gardneri* was the predominant species in the northern region while *X. perforans* was more prevalent in the southern region. In central Illinois, the distribution of *X. perforans* and *X. gardneri* was 55 and 45%, respectively. A previous study conducted in Ohio showed that *X. gardneri* and *X. perforans* were both present in tomato production fields (Ma et al., 2011). Other studies performed in cooler climate regions have reported *X. gardneri* as the dominant species (Cuppels et al., 2006; Kim et al., 2010). Likewise, it was shown that *X. gardneri* could causes more disease

with tomato plants grown at 20℃ in comparison to other *Xanthomonas* spp. (Araujo et al., 2010). This might explain the prevalence of *X. gardneri* in the northern region of Illinois, where the temperature is cooler compared to central and southern regions of the state. A recent study detected the presence of *X. perforans* in southern Ontario, Canada (Abbasi et al., 2015), which has relatively a cooler climate. It is unclear whether genetic differences exist between populations of *X. perforans* that might explain their ability to cause disease under different temperature conditions. In our study, the populations of *X. perforans* in Illinois are genetically similar to those found in Florida, which has a warmer climate (Potnis et al., 2011).

Multilocus sequence analysis based on six housekeeping genes revealed both endemic populations and genetically distinct isolates of *X. gardneri* and *X. perforans* in Illinois. The widespread occurrence of genetically similar isolates likely originated from recurring infections caused by infected plant materials prior years that spreads between nearby farms, or via contaminated materials shared between production sites. Additionally, the presence of multiple genetic groups of *X. perforans* has been reported in various tomato-growing regions such as North Carolina and Florida (Adhikari et al., 2019; Timilsina et al., 2015; Timilsina et al., 2019). About 68% of *X. perforans* isolates in our study grouped with *X. perforans* 91-118 (a reference isolate), suggested endemic dispersal of this genetic group. Alternatively, 27% of *X. perforans* isolates were grouped with *X. perforans* Xp-FL2 strains (Timilsina et al., 2015), but these were isolated from a single farm that also had a low number of Xp-IL1 strains, which suggests initial infection from different source that, likely over a period of some years, led to the endemic presence of Xp-IL2 strains at this particular farm.

Alternatively, localized and genetically distinct *X. gardneri* populations likely originated from infected seeds. Previous studies found that a single genetic strain of *X. gardneri* has been distributed around the world (Kebede et al., 2014; Timilsina et al., 2015). The two most diverse

genetic groups of *X. gardneri*, Xg-IL4 and Xg-IL5, were found to have nucleotide differences in *lepA* or *gltA* and *lepA* genes, respectively. Two groups with a small number of *X. perforans* isolates, Xp-IL3 and Xp-IL4, had nucleotide differences in *gap-1* or *gltA* and *lepA* genes, respectively. These smaller groups of *X. gardneri* and *X. perforans* strains were isolated from single farms and during one growing season, suggesting that the initial bacterial inoculum likely originated from different sources that were introduced to the individual farms.

In this study, *Xanthomonas* and non-*Xanthomonas* bacteria were isolated from symptomatic tomato foliage and fruits. Representative *Xanthomonas* isolates were pathogenic on eight-week-old tomato leaves, but none of the representative non-*Xanthomonas* isolates caused disease symptoms. We hypothesized that the non-*Xanthomonas* bacteria isolated from tomato foliage and fruits are likely saprophytes; however, our findings do not preclude the possibility that some of these bacteria may act synergistically with *Xanthomonas* to cause greater disease symptoms, or alternatively may be antagonistic to *Xanthomonas* pathogens.

This is the first study to assess and identify the causal agent for bacteria spot disease of tomato in Illinois. Identification of pathogens have been a driving factor in the development of resistant cultivars for disease management. Since all the commercially grown tomato cultivars were found to be susceptible to bacterial spot, future research should focus on developing more effective management strategies, including cultural practices and chemical use, for managing bacterial spot disease in Illinois conditions.

TABLES AND FIGURES

Table 2.1. Plant samples collected from symptomatic foliage and fruits from different regions of Illinois during 2017-2019

Region	Number of farms (number of fields)	Number of samples collected	
		Foliage	Fruit
Northern[x]	3 (9)	59	18
Central[y]	3 (6)	28	3
Southern[z]	3 (12)	70	9

[x] Northern region included two farms in McHenry County and one farm in Kane County.

[y] Central region included two farms in Douglas County and one farm in Moultrie County.

[z] Southern region includes three farms in Fayette County.

Table 2.2. Severity of bacterial spot symptoms on foliage in commercial tomato fields located in different regions of Illinois

	Disease severity (%)[y]								
	2017			2018			2019		
Region	Lowest	Highest	Ave.[z]	Lowest	Highest	Ave.[z]	Lowest	Highest	Ave.[z]
Northern	4.5	18.5	12	37.5	90.5	64	0	88	46
Central	3	4.5	2	9	18.5	10	5	75	55
Southern	9	37.5	20	9	81	50	45	92	70

[y] Percent affected plant area at the end of the growing season.

[z] Average disease severity.

Table 2.3. Incidence of tomato fruits with bacterial spot symptoms in commercial fields in Illinois

	Symptomatic fruits (%)		
Region	**2017**	**2018**	**2019**
Northern	<10	10-25	15-25
Central	0	0	20-30
Southern	<5	10-20	<5

Table 2.4. Number of *Xanthomonas* isolates collected from different regions of Illinois

	Foliage[z]		**Fruit**	
Regions	***X. gardneri***	***X. perforans***	***X. gardneri***	***X. perforans***
Northern	54	9	24	4
Central	13	17	4	2
Southern	34	94	4	7

[z] Isolated from all above-ground plant parts excluding fruits

Table 2.5: Number of *Xanthomonas* isolates with the different genetic groups in regions and by farm in Illinois

Species and genetic groups (no. of isolates)	Northern			Central			Southern		
	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3
X. perforans Xp-IL1 (90)	-	5	3	9	10	-	56	2	5
X. perforans Xp-IL2 (36)	-	-	-	-	-	-	-	36	-
X. perforans Xp-IL3 (2)	-	-	-	-	-	-	2	-	-
X. perforans Xp-IL4 (5)	5	-	-	-	-	-	-	-	-
X. gardneri Xg-IL1 (119)	18	38	8	11	-	6	22	16	-
X. gardneri Xg-IL2 (3)	-	3	-	-	-	-	-	-	-
X. gardneri Xg-IL3 (6)	6	-	-	-	-	-	-	-	-
X. gardneri Xg-IL4 (2)	2	-	-	-	-	-	-	-	-
X. gardneri Xg-IL5 (3)	3	-	-	-	-	-	-	-	-

Table 2.6: Non-*Xanthomonas* bacteria isolated from diseased tomato foliage and fruits

Bacterial genus	Number of isolates		
	2017	2018	2019
Agrobacterium	0	5	5
Bacillus	4	9	5
Curtobacterium	3	7	6
Microbacterium	10	82	15
Paenibacillus	12	1	5
Pantoea	0	18	35
Pseudomonas	0	56	35
Rhizobium	0	7	4
Stenotrophomonas	0	6	15
Miscellaneous	12	75	20
Total	41	226	145

Figure 2.1. County map of Illinois showing the counties surveyed to determine incidence of bacterial spot disease of tomatoes in commercial fields. The northern region included one farm in Kane County and two farms in McHenry County. The central region included two farms in Douglas County and one farm in Moultrie County. The southern region included three farms in Fayette County.

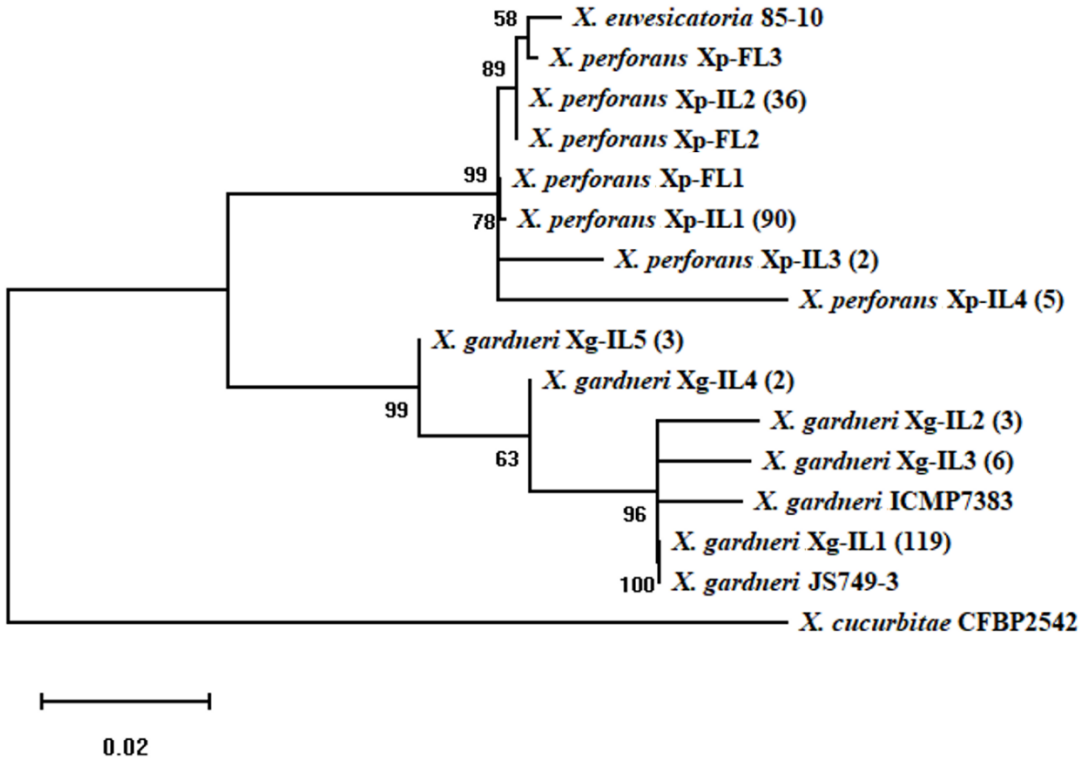

Figure 2.2. Multilocus sequence analysis using six housekeeping genes (*fusA, gap-1, gltA, gyrB, lepA*, *lacF*) distinguished five genetic groups for *Xanthomonas gardneri* and four groups for *X. perforans*. *Xanthomonas* isolates labeled as 'IL' were isolated from Illinois during 2017-2019. *X. perforans* labeled as 'FL' were defined as genetic groups in a previous study (Timilsina et al., 2015), while genetically distinct *X. gardneri* strains were used as references (Richard et al., 2017). IL genetic groups were defined as having less than 10 different bases over the total 3,092 nucleotides included in the analysis. The number of isolates present in each group is shown in parentheses. The scale bar represents the number of substitutions per site and values on the branches indicate Bayesian posterior probabilities expressed as the percentage of trees based on 1,000 bootstrap replicates.

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CHAPTER 3: EFFECTIVENESS OF CHEMICAL COMPOUNDS AND BIOPESTICIDES FOR MANAGEMENT OF BACTERIAL SPOT DISEASE OF TOMATO

ABSTRACT

Field and laboratory studies were conducted to determine the effectiveness of selected chemical compounds and biopesticides for management of bacterial spot disease of tomato in commercial fields in Illinois. Field trials were conducted using 'Mt. Fresh' and 'Red Deuce' tomatoes in 2017, 2018, and 2019. Using a back-pack sprayer, selected chemical compounds and biopesticides were sprayed on plants from the beginning the bloom stage until two weeks prior to final fruit harvest. Disease severity was higher on 'Red Deuce' than on 'Mt. Fresh' plants. Plots treated with copper hydroxide (Kocide-3000 46.1DF) plus mancozeb (Manzate PRO Stick) had the lowest disease severity on foliage of both cultivars. Only a few fruits in untreated plots developed bacterial spots toward the end of the growing seasons. Laboratory tests identified copper resistant isolates of *Xanthomonas perforans* and *X. gardneri* from around Illinois. PCR analysis indicated that the copper resistant isolates had two of the copper resistance genes, *copA* and *copM*. In addition, *in vitro* growth inhibition assays revealed that Kocide-3000 46.1DF alone or in combination with Manzate PRO Stick produced the largest inhibition zones for the copper sensitive isolates, while the combination of *Bacillus amyloliquefaciens* strain D747 (Double Nickel LC) and copper octanoate (Cueva) produced the largest inhibition zone for the copper resistant isolates. Together, these results indicate current chemical management practices may not provide satisfactory management of bacterial spot of tomato.

INTRODUCTION

Bacterial spot, caused by *Xanthomonas* spp., is one of the most important diseases of tomatoes in Illinois (Babadoost and Yu, 2017). Bacterial spot of tomato is a disease caused by four species: *X. euvesicatoria* (group A), *X. vesicatoria* (group B), *X. perforans* (group C), and *X. gardneri* (group D) (Jones et al., 2000; Jones et al., 2004).

Management of bacterial spot disease of tomato is very challenging task, mainly due to the lack of effective bactericides (Marco and Stall, 1983). Additional challenges include the development of copper resistant strains (Bender et al., 1990), the epiphytic survival of the bacteria on non-host plants (Sharon et al., 1982; Zhang et al., 2009), and the lack of effective resistant cultivars. Altogether, these factors can lead to significant crop losses, sometimes greater than 50%.

The predominant methods for managing bacterial spot of tomato are applications of copper compounds and antibiotics (Dougherty, 1978; Jones et al., 1991). Due to their low cost and minimal human toxicity, copper compounds have been popular for managing bacterial diseases for more than one century (Areas et al., 2018; Marco and Stall, 1983). However, excessive use of copper compounds has led to the development of copper resistant strains, including strains of *Xanthomonas campestris* pv. *vesicatoria* that were first reported in the early 1980s in Florida (Marco and Stall, 1983), and later reported on pepper and tomato fields in Oklahoma and North Carolina (Bender et. al., 1990; Ritchie and Dittapongpitch, 1991). Similarly, copper resistant *X. campestris* pv. *vesicatoria* strains have been found on pepper in Australia (Martin, et al., 2004), *X. euvesicatoria* on pepper in Brazil (Areas et al., 2018), and *X. perforans* on tomato in Canada (Abbasi et al., 2015).

The effectiveness of copper compounds is dependent on the concentration of free copper ions. However, when the ions are chelated by organic compounds such as those found in most crop production fields, they lose their toxicity to the bacteria (Menkissoglu and Lindow, 1991;

Zevenhuizen et al., 1979). Investigations have shown increased effectiveness of copper compounds against bacterial plant pathogens when used in combination with ethylene bisdithiocarbamate fungicides, such as mancozeb (Marco and Stall, 1983; Parsons and Edgington, 1981). Similarly, it has been reported increased control of *Xanthomonas campestris* pv. *juglandis* when iron was added to fixed copper-compounds like Kocide 101 (77% copper hydroxide) (Lee et al., 1993).

The copper-resistance phenotypes for two *Xanthomonas camprestris* pv. *vesicatoria* strains was discovered to be encoded by the self-transmissible plasmids; pXvCu (Stall et al., 1986) and pXV10A (Bender et al., 1990). Basim et al. (2005) characterized the *Xanthomonas campestris* pv. *vesicatoria* strain XvP26, originally isolated from pepper plants in Taiwan, as containing a 7,652 bp region, which was identified as the copper-resistance locus. De La Iglesia et al. (2010) designed first PCR primers to detect bacterial copper-resistance genes, specifically through amplification the copper P-type ATPases genes. Behlau et al. (2011) identified eight opening reading frames (ORFs) in the copper operon of *X. citri* subsp. *citri* A44 related to the copper operon genes *copL, copA, copB, copM, copG, copC, copD,* and *copF*.

The goal of our research is to study the effectiveness of chemical compounds and biopesticides in management of bacterial spot pathogens in Illinois. The aims of this study was: (i) to assess the effectiveness of selected chemical compounds and biopesticides for managing bacterial spot of tomato in the fields; and (ii) to determine if copper resistant *Xanthomonas* spp. isolates are present in tomato fields in Illinois. These findings will provide insight in future development of better management of bacterial spot of tomatoes in future.

MATERIALS AND METHODS

Field Trials.

During 2017-2019, field trials were conducted to evaluate the effectiveness of selected chemical compounds and biopesticides (Table 3.1) for managing bacterial spot of tomato caused

by *X. perforans* and *X. gardneri*. The trials were conducted in Champaign and Fayette Counties in Illinois.

2017 Trial. In 2017, a trial was conducted in a commercial field near the town of Shobonier (Fayette County) (Table 3.2). The soil was a silt clay with pH 6.2. The trial site was planted to oat cover crop in 2016. Soil was tilled on 14 April. Nitrogen fertilizer (135 kg/ha) was broadcast and incorporated on 14 April. Seeds of tomato 'Mt. Fresh' and 'Red Deuce' were planted in 48-cell trays containing steamed soil mix (soil:sand:peat; 1:1:1) in a greenhouse on 4 April. Six-week-old seedlings were transplanted into raised beds with drip irrigation and black plastic mulch on 16 May. The experiment was performed in a randomized complete block design with four replications, each with 10 plants in a single row spaced 69 cm apart. There were five plants of each cultivar in each plot (represents one replicate). Seedlings of 'Mt. Fresh' were transplanted on the west side of the plots and seedlings of 'Red Deuce' were on the east side of the plots. Plots were spaced 3 m apart within the row with 1.83 m centers between rows. Weeds were managed by hand during the season. Plants were irrigated and fertilized as needed through drip irrigation lines placed under the black plastic mulch.

Applications of chemical compounds and biopesticides began on 23 June and ended on 25 August, at 7 day intervals. Compounds were applied using a backpack sprayer with 469 L of solution per ha. The severity of bacterial spot symptoms on foliage (percent area of foliage affected) and incidence of the disease on fruits (percent fruit affected) were visually assessed for cultivar using all five replicate plants in each plot on the following dates: 6, 13, 20, and 27 July; 3, 10, 17, 24, and 31 August; and 7 September.

2018 Trial. In 2018, a trial was conducted at the University of Illinois Vegetable Research Farm in Champaign, Illinois (Table 3.3). The soil was a silt clay loam with pH 6.5. Soil was chisel-plowed on 2 December 2017 after soybeans had been harvested. Nitrogen fertilizer (67 kg/ha) was

broadcast and incorporated on 16 May 2018. Soil was tilled on 22 May to control weeds. Beds with drip irrigation tape were made on 25 May and covered with black plastic. The beds were 1.83-m apart, center to center. Seeds of tomato 'Mt. Fresh' and 'Red Deuce' were planted on April 12 in a greenhouse, as described for 2017 trial. Seedlings were transplanted on 25 May. The experiment was performed in a randomized complete block design with four replications, each with 10 plants in a single row spaced 70 cm apart. There were five plants of each cultivar in each plot (represents one replicate), as described in 2017 trial. Plots were spaced 4.57 m apart within the row. Weeds were managed by hand during the season. Plants were irrigated as needed through a drip irrigation system.

Applications of the chemical compounds and biopesticides began on 27 June and ended on 15 August, at 7 day intervals. Spray application of the compounds were as described for the 2017 trial. Severity of bacterial spot disease on leaves and incidence of bacterial spot on fruits were visually assessed on all plants on the following dates: 4, 11, 18, 25, and 31 Aug; and 7 Sep.

2019 Trial. In 2019, a trial was conducted at the same farm near Shobonier (Fayette County) where 2017 trial was carried out (Table 3.4). The trial site was planted to zucchini in 2018 and then was planted to a rye cover crop on 15 October 2018. Soil was tilled and fertilizers (67, 28, and 224 kg/ha of N, P, and K, respectively) were broadcast and incorporated on 10 May 2019. Beds with drip irrigation tape were made on 15 May and covered with black plastic. Seeds of tomato 'Mt. Fresh' and 'Red Deuce' were planted in a greenhouse on 10 April and seedlings were transplanted on 15 May. The experiment was performed in a randomized complete block design with four replications, as described for the trial in 2017 trial. Plants were irrigated and fertilized as needed.

Spray applications of chemical compounds and biopesticides began on 15 June and ended on 24 August, at 7 day intervals. Severity of bacterial spot on foliage and incidence of fruits with the

disease were visually assessed on the following dates: 12, 19, and 26 Jul; and 2, 9, 16, 23, and 30 Aug.

Laboratory Studies

Copper sensitivity of *Xanthomonas* isolates. *Xanthomonas* isolates collected from fields were tested for their copper sensitivity. *Xanthomonas* isolates were first cultured on Nutrient Agar (NA) in Petri plates and the plates were incubated at 28°C for 48 h. Then, the colonies were transferred onto Mannitol-Glutamate-yeast (MGY) agar amended with 0.8 mM copper sulfate in Petri plates (Behlau et al., 2013). The plates were incubated at 28°C for 48 h.

Determining the presence of copper-resistance genes in *Xanthomonas* isolates. *Xanthomonas* isolates that developed colonies on MGY agar were screened for the presence of the copper resistance genes *copA* and *copM* (Altimira et al., 2012; Behlau et al., 2011; Behlau et al., 2012) using a PCR assay. The PCR amplification of target genes were performed using a Pro-Flex thermal cycler (Thermo Fisher Scientific, Waltham, MA). A 25 μl reaction volume was prepared, which contained 2.5 μl of 10X PCR buffer, 0.5 μl of 10 mmol/μl of each primer, 0.5 μl of dNTPs, 19.875 μl of nuclease-free water, 0.125 μl of Taq polymerase and a small amount of bacteria (Behlau et al., 2012). PCR reactions were performed with initial denaturation at 95˚C for 5 min; 35 cycles of denaturation at 95˚C for 30 s, annealing at 55˚C for 30 s, and extension at 72˚C for 45 s; a final extension at 72˚C for 5 min, and then maintained at 4˚C. The PCR product was run in 1% agarose gel containing EZ-vision DNA dye (VWR Life Sciences, OH) at 100 V for 30 min and visualized at 470 nm using the Azure Biosystems c400 imager (Dublin, CA).

***In vitro* evaluation of the effects of copper compounds on multiplication of copper resistant *Xanthomonas* isolates.** A total of 11 copper resistant isolates were used in this study. The isolates had been collected during 2018 and 2019, with one representative isolate from each Illinois region (northern, central, and southern regions) for both *X. perforans* and *X. gardneri*. The

isolates were grown on Luria-Bertani (LB) agar at 28˚C for 48 h. Bacterial colonies were washed with sterile distilled water (SDW) and bacterial suspension with 5 x 10^8 CFU/ml (OD_{600}= 0.3). A drop (5 μl) bacterial suspension was placed on MGY agar amended with laboratory grade copper hydroxide and copper sulfate, at the following concentrations: 0.2, 0.8, 1.2, 1.6, and 2.0 mM. The plates were incubated at 28˚C for 48 h (Behlau et al., 2013) before evaluation of bacterial growth.

In addition, three commercial copper compounds were tested for their effects on multiplication of the bacterial isolates: copper oxychloride and copper hydroxide (Badge SC) at 1.88, 3.74, and 4.50 ml/L; copper hydroxide (Kocide-3000 46.1 DF) at 2.40, 3.69, and 4.19 g/L; and copper sulfate pentahydrate (Instill) at 256, 384, and 512 ml/L. Selected isolates were grown on LB agar at 28˚C for 48 h. Bacterial colonies were washed with SDW and a bacterial suspension with 5 x 10^8 CFU/ml (OD_{600}=0.3) was prepared and mixed with warm (unsolidified) LB agar at a ratio of 1:19 (v:v; bacterial suspension:LB agar). Approximately 20 ml of the LB agar/bacteria mixture was poured into each Petri plate. One filter paper circle (5 mm diameter) was soaked in each of the copper compounds dissolved in SDW and placed on the surface of each LB agar/bacteria plate. The plates were incubated at 28˚C, and after 48 h of incubation, the inhibition zones of bacterial reproduction were measured (Bauer et al., 1966; Fernando et al., 2005; Loper et al., 1991, Russo et al., 2008; Thapa, 2014).

***In vitro* evaluation of the effects of the selected compounds on multiplication of *X. perforans* and *X. gardneri*.** Identified copper-sensitive and copper resistant isolates of *X. perforans* and *X. gardneri* were evaluated for their multiplication on Luria-Bertani (LB) agar. Selected isolates were grown on LB agar at 28˚C for 48 h. Bacterial colonies were washed with sterile distilled water (SDW) and bacterial suspension with 5 X 10^8 CFU/ml ($OD_{600=}$ 0.3) was prepared and mixed with LB agar at the ratio of 1:19 (v:v; bacterial suspension:LB agar). Approximately 20 ml of the mixed LB agar with bacteria was poured into each Petri plate. A 5-

mm diameter filter paper soaked in the in the above-mentioned bactericide was placed on the surface of the LB agar with bacteria. The plates were incubated at 28˚C. Inhibition zones for bacterial reproduction were measured after 48 h of incubation (Bauer et al., 1966; Fernando et al., 2005; Loper et al., 1991, Russo et al., 2008; Thapa, 2014).

Data analysis. The data were analyzed using SAS 9.4 (SAS Institute Inc. Cary, NC). Data of the field trials were analyzed using the PROC GLM procedure to determine the effect of fixed treatments at $\alpha = 0.05$. Fisher's Protected Least Significant Difference (LSD) test was used to compare differences between the treatments. Type I error was controlled at $\alpha = 0.05$. Data from the field trials were analyzed separately because locations of the trials and compounds used were different for each trial. The data of laboratories studies were natural log transformed to meet the assumption of normality and homogeneity. PROC GLIMMIX was used to determine the effects of two factors (isolates and compounds) at $\alpha = 0.05$. LS means at $\alpha = 0.05$ were used to compare the differences between the treatments.

RESULTS

Field Trials

2017 Trial. Bacterial spot disease was observed in the plots throughout the growing season. The disease was first observed on 17 July, and the disease severity increased as the season progressed. Disease severity for 'Red Deuce' tomato was higher than that for 'Mt. Fresh' (Table 3.2). On 27 July, disease severity on foliage ranged from 0.0 to 0.8% for 'Mt. Fresh' and from 1.5 to 8.3% on for 'Red Deuce'. On August 17, disease severity on foliage ranged from 1.3 to 8.5% for 'Mt. Fresh' and 6.5 to 20.5% for 'Red Deuce'. On 7 September, the disease severity on foliage ranged from 4.8 to 25.5% for 'Mt. Fresh' and 19.5 to 46.3% for 'Red Deuce'. The total incidence of fruits with bacterial spot symptoms ranged from 0.0 to 0.5% for 'Mt. Fresh' and from 0.0 to 0.8% for 'Red Deuce'.

For both cultivars, disease severity on foliage was higher for untreated plots compared to the treated plots. Disease severity on foliage was the lowest in the plots of 'Mt. Fresh' treated with copper sulfate pentahydrate (Phyton Spectrum XP) alternated weekly with mancozeb (Manzate PRO Stick) plus copper hydroxide (Kocide-3000 46.1 DF). The severity of bacterial spot symptoms on foliage was less than 10% for 'Mt. Fresh' and 25% for 'Red Deuce' on 7 September (two weeks prior to the final harvest) in plots treated with quinoxyfen (Quintec 2.08SC) plus Kocide-3000 46.1 DF alternated with Manzate PRO Stick plus Kocide-3000 46.1 DF; azoxystrobin (Quadris 2.08SC) plus Kocide-3000 46.1 DF alternated with chlerothalonil (Bravo Weather Stik 6F) plus Kocide-3000 46.1 DF; Extract of *Reynoutria sachalinensis* plant (Regalia) alternated with Manzate PRO Stick plus Kocide3000 46.1 DF; and Phyton Spectrum XP alternated with Manzate PRO Stick plus Kocide3000 46.1 DF (Table 3.2).

On 7 September (two weeks prior to the final harvest), severity of bacterial spot symptoms on foliage for 'Mt. Fresh' was significantly lower ($P = 0.05$) in all treated plots compared to untreated plots. In comparison, there was no significant difference in severity of bacterial spot symptoms on foliage between treated and untreated plots of 'Red Deuce' (Table 3.2).

2018 Trial. In 2018, bacterial spot symptoms were observed first on foliage on 31 July, and disease severity increased as the season progressed (Table 3.3). Disease severity for 'Red Deuce' foliage was higher than for 'Mt. Fresh' foliage. On 4 and 18 August, disease severity in all treated plots was significantly lower compared to control plots for both cultivars. During the season, severity of bacterial spot on foliage for control plots of 'Mt. Fresh' increased up to 17.5%, whereas severity of the disease on foliage for control plots of 'Red Deuce' was 31.5% on 7 September (two weeks prior to final harvest). Application Manzate PRO stick plus Kocide-3000 46.1 DF, alternated with an extract of *Reynoutria sachalinensis* plant (Regalia) plus Kocide-3000 46.1 DF,

provided the best protection for ‘Mt. Fresh’ and ‘Red Deuce’ foliage (Table 3.3). No symptoms of bacterial spot were observed on fruits (i.e. 0% disease incidence for both cultivars).

2019 Trial. Only bacterial spot was observed in the plots throughout the growing season. Symptoms of the disease on foliage were first observed on 12 July for some plots of ‘Red Deuce’ and on 26 July for some plots of ‘Mt. Fresh’, and the disease severity increased as the season progressed (Table 3.4). Overall, severity of bacterial spot on foliage of ‘Red Deuce’ was higher than that of ‘Mr. Fresh’. On 19 July, disease severity on foliage was 0.0% for ‘Mt. Fresh’ and from 0.0 to 1.0% on for ‘Red Deuce’. On 9 August, disease severity on foliage ranged from 0.50 to 3.75% for ‘Mt. Fresh’ and 1.75 to 9.50% for ‘Red Deuce’. On 30 August, the disease severity on foliage ranged from 7.00 to 28.75 % for ‘Mt. Fresh’ and 20.75 to 55.00 % for ‘Red Deuce’. Severity of the disease in the plots that were treated with Manzate PRO Stick plus Kocide-3000 46.1 DF; chlerothalonil (Bravo Weather Stik) plus Kocide-3000 46.1 DF; quinoxyfen (Quintec 2.08 SC) plus Kocide-3000 46.1 DF alternated with Bravo Weather Stik plus Kocide-3000 46.1 DF; Regalia alternated with Bravo Weather Stik plus Kocide-3000 46.1 DF; Bacteriophage (AgriPhage); *Bacillus mycoides* isolate J (LifeGard) alternated with Bravo Weather Stik plus Kocide-3000 46.1 DF; and LifeGard plus Bravo Weather Stik plus Kocide-3000 46.1 DF was significantly lower than that of untreated control plots. Disease severity on foliage was consistently lowest for plots treated with Manzate PRO Stick plus Kocide-3000 46.1 DF (Table 3.4).

Severity of the disease in the untreated plots was significantly higher ($P = 0.05$) than those of treated plots on both ‘Mt. Fresh’ and ‘Red Deuce’. Disease severity on foliage was consistently lowest for plots treated with Manzate PRO Stick plus Kocide-3000 46.1 DF (Table 3.4). The only symptomatic fruits were observed in untreated control plots of ‘Red Deuce’ at the end of the season.

Laboratory Studies

Copper resistance status of *Xanthomonas* isolates. In 2018, a total of 158 isolates were collected, with 45 classified as copper resistant, as determined phenotypically by growth on media containing copper and genotypically by the presence of the copper resistance genes *copA* and *copM*. In the northern region, none of the tested *X. perforans* isolates were copper resistant, while copper resistant isolates of *X. gardneri* were detected in two of three farms (Table 3.5). In the central region, copper resistant isolates of *X. perforans* and *X. gardneri* were detected in one and two of the three farms, respectively. In the southern region, copper resistant isolates of *X. perforans* and *X. gardneri* were detected in two of the three farms.

In 2019, a total of 108 isolates were collected, with 83 classified as copper resistant, as determined phenotypically by growth on media containing copper. Additionally, a subset of strains were genotyped using PCR with the *copA* and *copM* primers. In the northern region, copper resistant isolates for *X. perforans* and *X. gardneri* were both present at the same two farms (Table 3.6). In the central region, only one of the three farms had copper resistant isolates of both *X. perforans* and *X. gardneri*. In the southern region, all three farms had copper resistant isolates of *X. perforans*; however, no *X. gardneri* isolates were obtained from the three farms (Table 3.6).

***In vitro* evaluation of the effects of copper compounds on multiplication of copper resistant *Xanthomonas* isolates.** Copper resistant *X. perforans* isolates were able to multiply when grown on media containing less than 2.0 mM of copper (either copper hydroxide or copper sulfate), while at 2.0 mM copper, all but two isolates were able multiply (Table 3.7). Similarly, copper resistant *X. gardneri* were able to multiple when grown on media containing less than 1.6 mM of copper, while at 1.6 and 2.0 mM of either copper hydroxide or copper sulfate, 80 and 100% of copper resistant of *X. gardneri* did not multiply (Table 3.7).

Among the commercial chemical compounds, filter papers soaked in the suspensions of Badge compound did not produce any inhibition zone on LB agar plates with the bacteria. Therefore, the related results were not presented. The results of the effects of Kocide-3000 46.1 and Instill on multiplication of the bacteria are provided in Tables 3.8 and 3.9. Both Kocide-3000 46.1 and Instill produced large inhibition zones in control plates (with copper-sensitive isolates) of both of *X. perforans* or *X. gardneri*.

Kocide-3000 46.1DF, with concentrations of 3.60 g/L and 4.19 g/L produced larger inhibition zone with copper resistant isolates of both *X. perforans* and *X. gardneri* compared to the concentration of 2.40 g/L (Tables 3.8 and 3.9). Instill, at any of the three concentration used did not produce inhibition zone with copper resistant *X. perforans* isolates (Tables 3.8). In contrast, Instill at concentrations of 384 ml/L and 512 ml/L produce inhibition zones with copper resistant isolates of *X. gardneri*, but no inhibition zone was produced at the concentration of 256 ml/L (Table 3.9).

***In vitro* evaluation of the effects of selected compound on multiplication of *X. perforans* and *X. gardneri*.** Manzate PRO Stick alone; Kocide-3000 46.1 DF plus Manzate PRO Stick; and *Bacillus amyloliquefaciens* strain D747 (Double Nickel) plus copper octanoate (Cueva) were significantly ($P < 0.05$) more effective on preventing multiplication of copper-sensitive isolates of both *X. perforans* (Figure 3.1) and *X. gardneri* (Figure 3.2). Double Nickel alone and Double Nickel plus Cueva was significantly ($P < 0.05$) more effective in preventing multiplication of copper resistant isolates of *X. perforans* compared to the other compounds tested (Figure 3.1). Manzate PRO Stick alone; Kocide-3000 46.1 DF plus Manzate PRO Stick; Double Nickel alone; and Double Nickel plus Cueva were significantly ($P < 0.05$) more effective in preventing multiplication of copper resistant isolates of *X. gardneri* compared to other compounds tested (Figure 3.2).

DISCUSSION

Bacterial spot is one of the most important tomato diseases in Illinois and throughout the US. Our field studies showed that all of the commercially grown tomato cultivars in Illinois were susceptible to bacterial spot disease, specifically caused by *X. perforans* and *X. gardneri*. Major recommendation for management of bacterial spot of tomato has included the application of copper compounds (Jones et al., 1991). However, several reports have indicated the development of copper resistant strains for several *Xanthomonas* species pathogenic on tomato and other crops (Abbasi et al., 2015; Areas et al., 2018; Bender et al., 1990; Marco and Stall, 1983; Martin et al., 2004). Our studies demonstrated that bacterial spot disease occurred in the plots that had been treated with copper compounds. In addition, copper resistant isolates for both *X. perforans* and *X. gardneri* were detected in major tomato production areas in northern, central, and southern Illinois.

Different chemical compounds and biopesticides were tested for their efficacy in managing bacterial spot disease present on 'Mt. Fresh' and 'Red Deuce' tomato plants grown in Fayette and Champaign Counties. Consistently, the disease severity was higher in 'Red Deuce' as compared to 'Mt. Fresh'. This suggests that there is genetic variation in susceptibility to bacterial spot between different tomato cultivars. However, producers will only grow tomato cultivars that have a market demand due to consumer preferences. Thus, developing strategies with integrated approaches for management of bacterial spot of tomatoes in field production is necessary.

Effective disease management of tomato is multifaceted and includes both genetic (e.g., the use of resistant cultivars) and environmental (e.g., cultural practices, chemical applications) components. At present, all cultivars of tomato grown in Illinois have shown susceptibility to bacterial spot disease (previous chapter), thus tomato growers have focused on controlling environmental factors. In particular, application of chemical compounds and biopesticides have been necessary for managing the disease. We evaluated most available potential compounds for

managing bacterial spot of tomato in Illinois. Specifically, Kocide-3000 46.1DF plus Manzate PRO Stick provided the best management of bacterial spot disease in all fields tested, which is in agreement with other reports that found copper compounds in combination with ethylene bisdithiocarbamate fungicides (i.e., mancozeb) are the most effective treatments for managing bacterial diseases of tomato plants (Jones and Jones, 1985; Marco and Stall, 1983; Parsons and Edginton, 1981). However, with development of copper resistant isolates of both *X. perforans* and *X. gardneri*, it is likely that even combination of Kocide-3000 46.1DF plus Manzate PRO Stick will not provide satisfactory protection of tomato plants against bacterial spot in Illinois. In addition, pre-harvest interval (PHI) of Manzate PRO Stick (mancozeb) is 5 days, which is not acceptable during the tomato fruit harvest period. There also have been previous report of copper resistant *Xanthomonas* strains and reports of decreased effectives of copper mancozeb combination to manage bacterial spot in tomato (Jones and Jones, 1985; Obradovic et al., 2005).

We evaluated bacterial growth in media containing 0.8 mM copper sulfate (threshold) and tested subsets of these isolates for the presence of the copper resistance genes *copA* and *copM*; bacteria that tested positive were considered copper resistant, in agreement with previous studies defining copper resistant isolates (Behlau et al., 2011; Behlau et al., 2013).

The presence of copper resistant isolates and the decreased efficacy of traditional approaches for control of bacterial spot of tomato have increased the necessity for alternative or complimentary control methods, such as the development of biopesticides. However, efficacy and availability of biopesticidies is limited (Byrne et al., 2005, Wilson et al., 2002). Our *in vitro* efficacy study comparing copper sensitive and copper resistant isolates of *X. perforans* and *X. gardneri* showed that the biopesticide, Double Nickel (Strain of *Bacillus amyloliquefaciens)* had similar suppression of both copper resistant and copper sensitive isolates; however, the standard copper-mancozeb combination treatment had reduced efficacy at reducing growth of copper resistant isolates

compared to copper sensitive isolates. Previous studies have also reported the use of several different biopresticides and their combination that resulted in similar or superior results compared to the standard copper-mancozeb combination (Louws et al., 2001; Obradovic et al., 2005). There are also have been attempts to integrate biopesticides and traditional chemical treatments to control of biopesticides and traditional chemical treatments to control of several other plant disease (Ji et al., 2006). With the presence of copper resistant isolates in Illinois tomato fields, use of the standard copper-based bactericide program may have reduced efficacy for managing bacterial spot of tomato. Further-research is needed to develop better management practices for control of bacterial spot of tomato in Illinois.

TABLES AND FIGURES

Table 3.1. Chemical compounds and biopesticides used for management of bacterial spot of tomato in Illinois during 2017-2019

Compound	Active ingredients	FRAC code[w]
Chemical compounds		
Badge SC	Copper oxychloride + copper hydroxide	M1[x]
Badge X2	Copper oxychloride + copper hydroxide	M1
Bravo Weather Stik 6F	Chlerothalonil	M5[x]
Cueva	Copper octanoate	M1
Instill	Copper sulfate pentahydrate	M1
Kocide-3000 46.1DF	Copper hydroxide	M1
Manzate PRO Stick	Mancozeb	M3[x]
NanoPro	Humic acid	NC
Phyton 27 AG	Copper sulfate pentahydrate	M1
Phyton Spectrum XP	Copper sulfate pentahydrate	M1
Quadris 2.08SC	Azoxystrobin	11
Quintec 250SC	Quinoxyfen	13
Biopesticides		
Agriphage	Bacteriophage	NC[y]
Double Nickel LC	*Bacillus amyloliquefaciens* strain D747	44
Lifegard WG	*Bacillus mycoidesi* isolate J	NC
Regalia	Extract of *Reynoutria sachalinensis*	P5[z]

[w] FRAC = Fungicide Resistance Action Committee.

[x] M1, M3, and M5: fungicides with multi-site contact activity.

[y] NC = not determined yet.

[z] P5 = Unique mode of action.

Table 3.2. Efficacy of selected chemicals and biopesticides for management of bacterial spot of tomatoes in Illinois in 2017

Treatment[u] and rate/ha (application timing)[v]	Severity of bacterial spot on foliage (%)[s]								Infected fruit (%)[t]	
	27 Jul		17 Aug		7 Sep		AUDPC[v]		7 Sep	
	Mt. Fresh[w]	Red Deuce[w]	Mt. Fresh	Red Deuce	Mt. Fresh	Red Deuce	Mt. Fresh	Red Deuce	Mt. Fresh	Red Deuce
Untreated check	0.0 b[y]	2.5 b	6.1 ab	20.5 ab	25.5 a	46.3 a	276.5 a	842.6 ab	0.0 b	0.8 a
Quintec, 0.42 L + Kocide, 1.68 kg (1,3,5,7,9) *alt*[z] Manzate, 1.68 kg + Kocide, 1.68 kg (2,4,6,8,10)	0.3 ab	3.3 ab	2.3 cd	10.8 a-c	4.8 d	24.3 a	93.6 cd	485.6 ab	0.0 b	0.0 a
Quadris, 0.45 L + Kocide, 1.68 kg (1,3,5,7, 9) *alt* BWS, 2.35 L + Kocide, 1.68 kg (2,4,6,8,10)	0.0 b	2.5 b	2.3 cd	8.3 bc	7.0 d	19.5 a	105.0 cd	395.5 b	0.0 b	0.5 a
Regalia, 2.35 L (1,3,5,7, 9) *alt* Manzate, 1.68 Kg + Kocide, 1.68 Kg (2,4,6,8,10)	0.8 ab	3.8 ab	1.3d	8.5 bc	6.0 d	24.0 a	77.9 d	451.5 ab	0.0 b	0.3 a
Cueva, 4.67 L + Double Nickel LC, 2.35 L (1,3,5,7, 9) *alt* Quintec, 0.42 L + Kocide, 1.68 kg (2,4,6,8,10)	0.0 b	1.8 b	2.3 cd	10.8 a-c	8.0 cd	25.3 a	89.3 d	455.9 ab	0.0 b	0.5 a
Phyton Spectrum XP, 2.91 L (1,3,5,7, 9) *alt* Manzate, 1.68 kg + Kocide, 1.68 kg (2,4,6,8,10)	0.3 ab	1.3 b	1.3 d	6.5 c	6.0 d	19.5 a	71.8 d	327.3 b	0.0 b	0.0 a
Phyton 27AG, 2.91 L (1-10)	0.8 a	1.8 b	4.5 bc	18.3 a-c	14.3 bc	31.0 a	196.9 a-c	619.5 ab	0.5 a	0.5 a

Table 3.2. Continued

Phyton Spectrum XP, 2.91 L (1-10)	0.0 b	1.5 b	3.5 b-d	17.8 a-c	8.5 cd	34.5 a	119.0 b-d	624.8 ab	0.3 ab	0.0 a
Regalia, 5.80 L (1-10)	0.8 ab	8.3 a	8.5 a	20.5 ab	15.8 b	40.0 a	296.6 a	978.3 a	0.0 b	0.5 a
Manzate, 1.68 kg + Kocide, 1.68 kg (1-10)	0.5 ab	4.8 ab	2.8 cd	16.3 a-c	6.0 d	35.8 a	106.8 cd	784.9 ab	0.0 b	0.5 a
Cueva SC, 4.67 L + Double Nickel LC, 2.35 L (1-10)	0.8 ab	7.8 a	5.0 bc	23.5 a	13.8 bc	42.5 a	223.1 ab	957.3 a	0.3 ab	0.3 a
LifeGard, 158 g (1,3,5,7,9) *alt* Manzate, 1.68 kg lb + Kocide, 1.68 kg (2,4,6,8,10)	1.0 a	5.5 ab	4.0 b-d	13.5 a-c	8.8 cd	28.3 a	160.1 b-d	576.6 ab	0.0 b	0.5 a
LSD (P = 0.05)	1.0	5.1	3.1	13.7	6.4	28.0	105.1	551.2	0.4	0.8

[s] Percent area of foliage affected.

[t] Infected fruit with *Xanthomonas* spp.

[u] Area Under the Disease Progress Curve.

[v] Treatments: BWS (Bravo Weather Stik 6F) = chlerothalonil; Cueva SC = Copper Octanoate; Double Nickel LC = *Bacillus amyloliquefaciens* strain D747; Kocide (Kocide-3000 41.6DF) = copper hydroxide; LifeGard = *Bacillus mycoides* isolate J; Manzate (Manzate PRO Stick) = mancozeb; Phyton 27AG = copper sulfate Pentahydrate; Phyton Spectrum XP = copper sulfate; Quadris (Quadris 2.08SC) = azoxystrobin; Quintec (Quintec 2.08SC) = quinoxyfen; and Regalia = an extract of *Reynoutria sachalinensis* plant.

[w] Tomato cultivars.

[x] Application date: 1 = 23 June, 2 = 30 June, 3 = 7 July, 4 = 14 July, 5 = 21 July, 6 = 28 July, 7 = 4 August, 8 = 11 August, 9 = 18 August, and 10 = 25 August.

[y] Values within each column followed with the same letter are not significantly different (P = 0.05) according to Fisher's Protected Least Significant Difference test.

[z] *alt* = Alternated with.

Table 3.3. Efficacy of selected chemical compounds and biopesticides for management of bacterial spot of tomato in Illinois in 2018

Treatment[v] and rate/ha (application timing)[w]	Severity of bacterial spot on foliage (%)[t]							
	4 August		18 August		7 September		AUDPC[u]	
	Mt. Fresh[x]	Red Deuce[x]	Mt. Fresh	Red Deuce	Mt. Fresh	Red Deuce	Mt. Fresh	Red Deuce
Untreated check	7.5 a[y]	7.5 a	10.0 a	15.0 a	17.5 a	31.2 a	376.2 a	629.1 a
Manzate, 1.68 kg + Kocide, 1.68 kg (1,3,5,7) *alt*[z] Regalia, 2.35 L + Kocide, 1.68 kg (2,4,6,8)	3.7 b	3.7 b	5.0 b	6.5 b	8.5 b	11.2 c	195.1 b	259.0 c
Badge SC, 2.35 L (1-8)	3.0 b	3.0 b	5.0 b	6.7 b	10.0 b	16.2 bc	211.8 b	304.5 bc
Badge X2, 2.25 kg (1-8)	3.7 b	3.7 b	5.0 b	7.2 b	8.0 b	15.0 bc	212.6 b	293.1 bc
GWN 10320, 2.35 L (1-8)	4.0 b	4.0 b	5.0 b	8.5 b	10.5 b	23.7 ab	229.2 b	431.3 b
GWN 10320, 2.35 L + Badge X2, 2.25 kg (1-8)	3.5 b	3.5 b	4.0 b	6.7 b	8.0 b	17.5 bc	178.5 b	324.5 bc
LSD ($P = 0.05$)	3.0	3.0	3.5	3.7	4.3	8.9	112.5	153.8

[t] Percent area of foliage affected.

[u] Area Under the Disease Progress Curve.

[v] Treatments: Badge SC = Copper oxychloride + copper hydroxide; Badge X2 = Copper oxychloride + copper hydroxide; GWN 10320 = an experimental compound; Kocide (Kocide-3000 41.6DF) = copper hydroxide; Manzate (Manzate PRO Stick) = mancozeb; and Regalia = an extract of *Reynoutria sachalinensis* plant.

[w] Application date: 1 = 27 June, 2 = 4 July, 3 = 11 July, 4 = 17 July, 5 = 25 July, 6 = 2 August, 7 = 8 August, and 8 = 15 August.

[x] Tomato cultivars.

[y] Values within each column followed with the same letter are not significantly different ($P = 0.05$) according to Fisher's Protected Least Significant Difference test.

[z] *alt* = Alternated with.

Table 3.4. Efficacy of selected chemical compounds and biopesticides for management of bacterial spot of tomato in Illinois in 2019

Treatment[v] and rate/ha (application timing)[w]	Severity of bacterial spot on foliage (%)[t]							
	19 July		9 August		30 August		AUDPC[u]	
	Mt. Fresh[x]	Red Deuce[x]	Mt. Fresh	Red Deuce	Mt. Fresh	Red Deuce	Mt. Fresh	Red Deuce
Untreated check	0.00	1.00 a[y]	3.00 a-c	9.50 a	28.75 a	55.00 a	242.38 a	584.50 a
Manzate, 1.68 kg + Kocide, 1.68 kg (1-11)	0.00	0.00 b	0.50 d	1.75 e	7.00 e	20.75 d	47.25 c	133.88 e
BWS, 2.35 L + Kocide, 1.68 kg (1-11)	0.00	0.00 b	0.75 d	3.25 de	8.75 de	25.00 cd	60.38 bc	185.50 de
Quintec, 0.42 L + Kocide, 1.68 kg (1,3,5,7,9,11) *alt*[z] BWS, 2.35 L + Kocide, 1.68 kg (2,4,6,8,10)	0.00	0.00 b	0.75 d	2.25 e	11.25 d-e	23.75 cd	70.88 bc	179.38 de
Regalia, 2.35 L (1,3,5,7,9,11) *alt* BWS, 2.35 L + Kocide, 1.68 kg (2,4,6,8,10)	0.00	0.25 ab	1.75 b-d	4.75 b-e	11.75 c-e	26.25 cd	116.38 b	242.38 d
AgriPhage, 2.35 L (1-11)	0.00	0.0 b	1.25 cd	4.50 c-e	12.50 c-e	21.75 cd	85.75 bc	198.63 de
LifeGard, 158 g (1,3,5,7,9,11) *alt* BWS, 2.35 L + Kocide, 1.68 kg (2,4,6,8,10)	0.00	0.00 b	1.75 b-d	4.25 c-e	15.00 b-d	32.50 b-d	115.50 b	260.75 cd
LifeGard, 158 g + BWS, 2.35 L + Kocide3000, 1.68 kg (1-11)	0.00	0.00 b	1.00 d	3.00 de	8.75 de	26.25 cd	67.38 bc	196.88 de
Instill, 1.48 L (1-11)	0.00	0.00 b	3.00 a-c	6.25 a-d	21.25 b	33.75 bc	184.63 a	347.38 bc
Instill, 1.48 L + NanoPro, 0.30 L (1-11)	0.00	0.50 ab	3.25 ab	7.00 a-c	18.75 bc	33.75 bc	193.38 a	359.63 bc

Table 3.4. Continued

Instill, 0.74 L (1-11)	0.00	0.75 ab	3.50 ab	8.00 ab	18.75 bc	32.50 b-d	216.13 a	406.00 b
Instill, 0.74 L + NanoPro, 0.30 L(1-11)	0.00	0.00 b	3.75 a	8.25 a	20.00 b	40.00 b	199.50 a	371.00 b
LSD (P = 0.05)	NS[v]	0.77	1.93	3.46	7.33	12.11	60.96	104.38

[t] Percent area of foliage affected.
[u] Area Under the Disease Progress Curve.
[v] Treatments: Agriphage = a bacterial virus; BWS (Bravo Weather Stik 6F) = chlerothalonil; Instill = Copper sulfate pentahydrate; Kocide (Kocide-3000 41.6DF) = copper hydroxide; LifeGard = *Bacillus mycoides* isolate J; Manzate (Manzate PRO Stick) = mancozeb; NanoPro = Humic acid; Quintec (Quintec 2.08SC) = quinoxyfen; and Regalia = an extract of *Reynoutria sachalinensis* plant.
[w] Application date: 1 = 23 June, 2 = 30 June, 3 = 7 July, 4 = 14 July, 5 = 21 July, 6 = 28 July, 7 = 4 August, 8 = 11 August, 9 = 18 August, and 10 = 25 August.
[x] Tomato cultivars.
[y] Values within each column followed with the same letter are not significantly different (P = 0.05) according to Fisher's Protected Least Significant Difference test.
[z] *alt* = Alternated with.

Table 3.5. Copper resistant isolates of *Xanthomonas perforans* and *X. gardneri* in commercial tomato fields in Illinois in 2018

Pathogen species (no. of isolates tested)	Region									
	Northern			Central			Southern			
	Farm-1	Farm-2	Farm-3	Farm-1	Farm-2	Farm-3	Farm-1	Farm-2	Farm-3	Farm-4[w]
X. perforans (56)	0[x]/5[y]	---[z]	---	3/7	0/5	---	9/18	5/21	---	---
X. gardneri (102)	9/30	5/25	0/3	1/3	---	2/3	7/22	5/16	---	---

[w] Farm 4 was not surveyed in 2018

[x] Number of copper resistant isolates detected.

[y] Total number of isolates tested.

[z] The pathogen did not occur in the field.

Table 3.6. Copper resistant isolates of *Xanthomonas perforans* and *X. gardneri* in commercial tomato fields in Illinois in 2019

Pathogen species (no. of isolates tested)	Region									
	Northern			Central			Southern			
	Farm-1	Farm-2	Farm-3	Farm-1	Farm-2	Farm-3	Farm-1	Farm-2	Farm-3[w]	Farm-4
X. perforans (77)	---[x]	2[y]/4[z]	1/4	9/9	0/10	---	26/26	19/19	---	5/5
X. gardneri (31)	---	8/13	5/8	8/8	---	0/2	---	---	---	---

[w] Farm 3 was not surveyed in 2019

[x] The pathogen did not occur in the field.

[y] Number of copper resistant isolates detected.

[z] Total number of isolates tested.

Table 3.7. Effects of copper compounds on multiplication of *Xanthomonas perforans* and *X. gardneri* from commercial tomato fields in Illinois on MGY agar

Year	Region	Bacterial isolate	Copper hydroxide (mM)[w]	Copper sulfate (mM)[w]
	Control[x]	*X. perforans*	++[y] 0.2	++ 0.2
		X. gardneri	++ 0.2	++ 0.2
2018	Northern	*X. perforans*	++ 1.6	+[z] 1.6
		X. gardneri	+ 1.6	+ 1.6
	Central	*X. perforans*	++ 1.6	++ 1.6
		X. gardneri	++ 1.6	++ 1.6
	Southern	*X. perforans*	++ 1.6	++ 1.6
		X. gardneri	++ 1.6	++ 1.6
2019	Northern	*X. perforans*	++ 1.6	++ 1.6
		X. gardneri	++ 1.2	++ 1.2
	Central	*X. perforans*	++ 1.6	++ 1.6
		X. gardneri	++ 1.6	++ 1.6
	Southern	*X. perforans*	++ 2.0	++ 2.0

[w] mM = millimolar.

[x] Copper sensitive isolates collected .

[y] ++ = Colonies developed within 48 hr.

[z] + = Colonies developed after 48 hr.

Table 3.8: *In-vitro* evaluation of the effects of copper compounds on multiplication of *Xanthomonas perforans* from commercial tomato fields in Illinois on Luria Bertani agar

Bacterial isolate (region-year)[u]	Inhibition zone (mm)						LSD
	Copper hydroxide (Kocide-3000 41.6DF)[s]			Copper sulfate pentahydrate (Instill)[t]			
	2.40 g/L[v]	3.60 g/L[v]	4.19 g/L[v]	(256 ml/L)[w]	(384 ml/L)[w]	(512 ml/L)[w]	(*P*=0.05)
Control[x]	8.88 a[y] (C)[z]	11.80 ab (A)	12.30 a (A)	10.23 a (B)	11.80 a (A)	12.30 a (A)	1.51
Nothern-2018	0.00 b (B)	12.20 a (A)	12.70 a (A)	0.00 b (B)	0.00 b (B)	0.00 b (B)	0.59
Nothern-2019	0.00 b (B)	12.15 a (A)	12.05 ab (A)	0.00 b (B)	0.00 b (B)	0.00 b (B)	0.66
Centra-2018	0.00 b (C)	10.25 c (B)	11.28 bc (A)	0.00 b (C)	0.00 b (C)	0.00 b (C)	0.33
Central-2019	0.00 b (C)	9.80 c (B)	11.08 bc (A)	0.00 b (C)	0.00 b (C)	0.00 b (C)	0.36
Southern-2018	0.00 b (B)	10.60 bc (A)	10.60 c (A)	0.00 b (B)	0.00 b (B)	0.00 b (B)	0.81
Southern-2019	0.00 b (C)	10.53 bc (A)	10.53 c (A)	0.00 b (C)	0.00 b (C)	0.00 b (C)	0.48
LSD (*P* = 0.05)	1.87	1.20	0.98	0.52	0.82	0.23	

[s] A commercial product of copper hydroxide for agricultural application.
[t] A commercial product of copper sulfate for agricultural application
[u] Region and year of collecting the isolate.
[v] Grams of commercial compound per liter of sterile-distilled water that the filter paper was soaked before placing on culture medium with the pathogen.
[w] Milliliters of commercial compound per liter of sterile-distilled water that the filter paper was soaked before placing on culture medium with the pathogen.
[x] A copper sensitive isolate of *X. perforans*.
[y] Values within each column followed with the lower case of the same letter are not significantly different (*P* = 0.05) according to Fisher's Protected Least Significant Difference test.
[z] Values within each row followed with the higher case of the same letter in the parentheses are not significantly different (*P* = 0.05) according to Fisher's Protected Least Significant Difference test.

Table 3.9: *In-vitro* evaluation of the effects of copper compounds on multiplication of *Xanthomonas gardneri* from commercial tomato fields in Illinois on Luria Bertani agar

Bacterial isolate (region-year)[u]	Inhibition zone (mm)						LSD (*P*=0.05)
	Copper hydroxide (Kocide-3000 41.6DF)[s]			Copper sulfate pentahydrate (Instill)[t]			
	2.40 g/L[v]	3.60 g/L[v]	4.19 g/L[v]	(256 ml/L)[w]	(384 ml/L)[w]	(512 ml/L)[w]	
Control[x]	9.63 a[y] (D)[z]	12.05 a (B)	13.95 a (A)	10.73 a (C)	12.28 a (B)	12.85 a (AB)	1.20
Nothern-2018	0.00 b (C)	10.08 b (B)	11.73 ab (A)	0.00 b (C)	10.33 b (B)	11.62 bc (A)	0.71
Nothern-2019	0.00 b (D)	10.08 bc (C)	11.73 ab (A)	0.00 b (D)	10.33 b (BC)	11.08 cd (BC)	0.93
Central-2018	0.00 b (C)	10.00 bc (B)	9.38 d (B)	0.00 b (C)	10.00 c (B)	12.20 ab (A)	0.85
Central-2019	0.00 b (B)	10.25 b (A)	10.75 bc (A)	0.00 b (B)	10.00 c (A)	10.63 d (A)	1.02
Southern-2018	0.00 b (C)	9.03 c (B)	10.45 cd (A)	0.00 b (C)	10.63 bc (A)	11.13 cd (A)	1.62
LSD (*P* = 0.05)	1.15	1.55	1.19	0.31	0.97	0.85	

[s] A commercial product of copper hydroxide for agricultural application.
[t] A commercial product of copper sulfate for agricultural application
[u] Region and year of collecting the isolate.
[v] Grams of commercial compound per liter of sterile-distilled water that the filter paper was soaked before placing on culture medium with the pathogen.
[w] Milliliters of commercial compound per liter of sterile-distilled water that the filter paper was soaked before placing on culture medium with the pathogen.
[x] A copper sensitive isolate of *X. gardneri*.
[y] Values within each column followed with the lower case of the same letter are not significantly different (*P* = 0.05) according to Fisher's Protected Least Significant Difference test.
[z] Values within each row followed with the higher case of the same letter in the parentheses are not significantly different (*P* = 0.05) according to Fisher's Protected Least Significant Difference test.

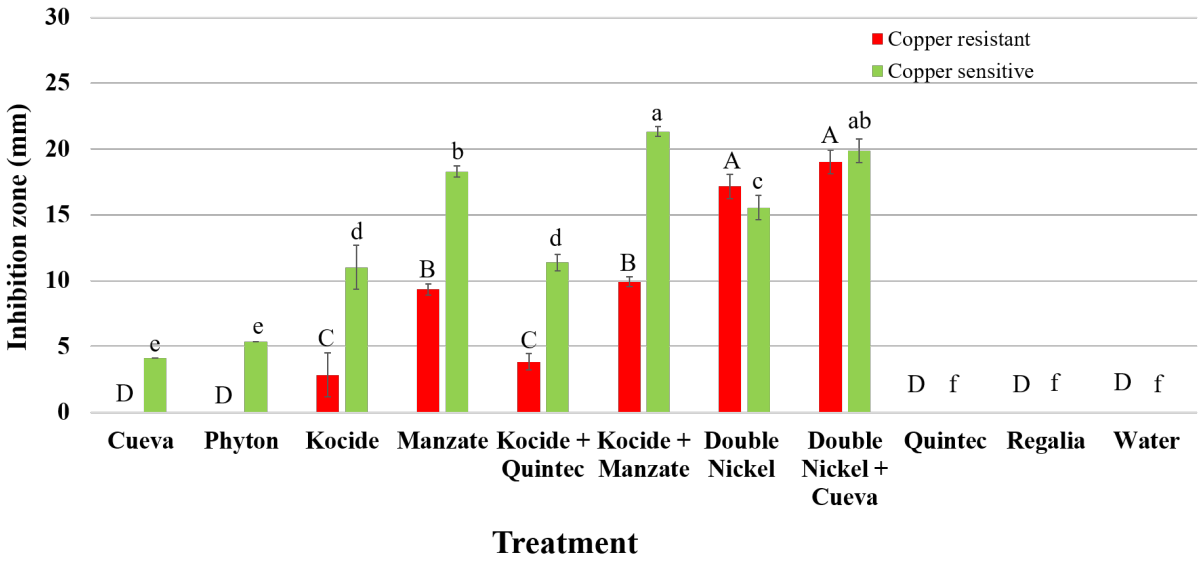

Figure 3.1. *In vitro* evaluation of the effects of chemical compounds and biopesticides on multiplication of copper -resistant and copper sensitive isolates of *Xanthomonas perforans*. Results shown are the means +/- standard deviation. Different letters indicate significant differences determined by Fisher's Protected Least Significant Difference test ($P < 0.05$); uppercase letters are used for the copper resistant isolates (red bars) while lowercase letters are used for the copper sensitive isolates (green bars). Treatments: Cueva = copper octanoate; Phyton = copper sulfate pentahydrate; Kocide (Kocide-3000 41.6DF) = copper hydroxide; Manzate (Manzate PRO Stick) = mancozeb; Quintec (Quintec 2.08SC) = quinoxyfen; Double Nickel = *Bacillus amyloliquefaciens* strain D747; and Regalia = an extract of *Reynoutria sachalinensis* plant.

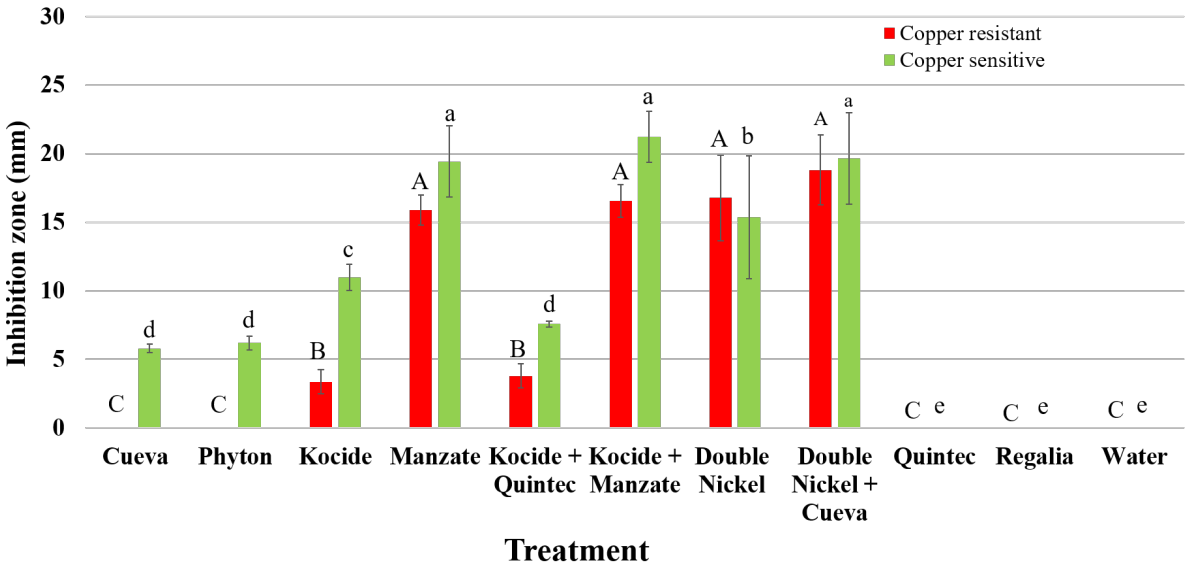

Figure 3.2. *In vitro* evaluation of the effects of chemical compounds and biopesticides on multiplication of copper resistant and copper-sensitive isolates of *Xanthomonas gardneri*. Results shown are the means +/- standard deviation. Different letters indicate significant differences determined by Fisher's Protected Least Significant Difference test ($P < 0.05$); uppercase letters are used for the copper resistant isolates (red bars) while lowercase letters are used for the copper sensitive isolates (green bars). Treatments: Cueva = copper octanoate; Phyton = copper sulfate pentahydrate; Kocide (Kocide-3000 41.6DF) = copper hydroxide; Manzate (Manzate PRO Stick) = mancozeb; Quintec (Quintec 2.08SC) = quinoxyfen; Double Nickel = *Bacillus amyloliquefaciens* strain D747; and Regalia = an extract of *Reynoutria sachalinensis* plant.

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