FIELD SURVIVAL OF *XANTHOMONAS CUCURBITAE*, THE CAUSAL AGENT OF BACTERIAL SPOT OF PUMPKIN, AND EFFICACY OF SELECTED CHEMICALS AND BIOCONTROL AGENTS FOR CONTROL OF THE DISEASES

BY

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THESIS

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ABSTRACT

Bacterial spot, caused by *Xanthomons cucurbitae*, has become one of the most important diseases of pumpkins in Illinois. This research was conducted to assess survival of *X. cucurbitae* in commercial fields, and to evaluate efficacy of selected chemicals and biocontrol agents for control of bacterial spot of pumpkin. To assess survival of *X. cucurbitae* in the field, a factorial experiment was setup at the University of Illinois Fruit and Vegetable Research Farm in Urbana, IL. Field location, plant tissue, burial depth, and recovery date were the experimental factors. The experimental unit was either five infected leaves or two infected fruit pieces (10 cm × 10 cm), which were placed in fiber glass mesh and buried either at 0-10 cm or 10-20 cm deep on 7 October 2011. The samples were recovered on 7 April, 7 July, and 7 October 2012; and 7 January, 7 April, and 7 October 2013 and processed for presence of *X. cucurbitae*. The location of the field did not significantly ($P = 0.7895$) affect the number of *X. cucurbitae* CFUs recovered from the samples. However, survival of *X. cucurbitae* was significantly affected by burial depths ($P = 0.08$), the plant tissue ($P = 0.001$) and time period of plant tissue in soil ($P = 0001$). *X. cucurbitae* survived for 24 months in both infected pumpkin leaf and fruit tissues in the soil. Seventeen chemicals and five biocontrol agents were tested in laboratory and field for their efficacy for control of bacterial spot. Ten isolates of *X. cucurbitae* were tested in nutrient broth (NB) and casitone yeast extract broth (CYE). Laboratory studies of chemicals were conducted to determine effective concentrations for 50% and 100% reduction in cell multiplication (EC_{50} and EC_{100}) of *X. cucurbitae*. The EC_{50} values of the chemicals ranged from 0.17 ppm mancozeb (Dithane) to 64.53 ppm cuprous oxide (Nordox) in NB, and from 0.23 ppm mancozeb (Dithane) to 38.87 ppm cuprous oxide (Nordox) in CYE. Similarly, the EC_{100} values of the chemicals ranged from 1 ppm mancozeb (Dithane) to 175 ppm cuprous oxide (Nordox) in NB, and from 1 ppm mancozeb (Dithane) to 125 ppm cuprous oxide (Nordox) in CYE. The Laboratory tests of

biocontrol agents were conducted using the filter-disc assay method. All five tested biocontrol agents prevented cell multiplication of *X. cucurbitae* cells around the filter disc dipped in biocontrol agents. The field trials were carried out at the University of Illinois Vegetable Research Farm in Champaign, IL. Jack-o-lantern pumpkin 'Howden' was used in the field studies. Plants were inoculated with an equally mixed inoculum of 10 *X. cucurbitae* isolates (5×10^7 CFU/ml). Spray-application of chemicals began either pre- or post-inoculation of plants. Spray-application of all biocontrol agents began pre-inoculation. All compounds were applied at 7-day intervals. Incidence and severity of bacterial spot in leaves were significantly lower in treated plots than those of untreated control plots. Treatments with Badge, Cuprofix, Mycoshield, Phyton, Kocide plus ActiGard, and Kocide plus Tanos were more effective in reducing incidence and severity of bacterial spot on both leaves and fruit than other chemicals. Treatments with biocontrols Regalia, Serenade, and Serenade plus Milstop were more effective in reducing incidence and severity of bacterial spot on both leaves and fruit than other biocontrol treatments.

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TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Cucurbitaceae family and pumpkin

The family cucurbitaceae includes some of the world's important vegetable crops, such as cucumber *(Cucumis sativus)*, gourd (*Lagenaria siceraria*), melon (*Cucumis melo*), pumpkins and squashes (*Cucurbita pepo*, *C. maxima*, and *C. moschata*, and *C. argyrosperma*), and watermelon (*Citrullus lanatus*) (Berg, 2008). This family has more than 700 species, including crops and weeds. Cucurbits crops are grown worldwide.

Pumpkin refers to certain cultivars of squash of any one of the species *Cucurbita pepo* L., *C. argyrosperma* K. Kock (syn.: *C. mixta*), *C. maxima* Duchesne, and *C. moschata* (Duchesne) Duchesne ex Poir (e.g., 'Dickenson' and 'Libby Select', known as processing pumpkin). The name pumpkin is derived from a Greek meaning word 'large melon' the French adopted it as 'pompon', which British changed to 'pumpion', and later Americans changed it to the name we use today, 'pumpkin'(Babadoost and Zitter, 2009; Michael et. al., 2012).

Pumpkins vary in shape (round, oblong to pear shaped), size (5 cm to more than 50 cm in diameter), weight (0.5 kg to 500 kg), and color (orange, white, yellow, and green) (Babadoost and Zitter, 2009). Pumpkins are monoecious, having both male and female flowers on the same plant. Flowers are usually yellow in color, unisexual, and female flowers are distinguished by the small ovary at the base of the petals. The color of the pumpkin is derived from the orange pigments abundant in female flowers. The main nutrients in pumpkin are lutein, and alpha and beta carotene, the latter of which generates vitamin A in the human body (Whitaker, 1950).

Pumpkin production

Pumpkins are grown throughout the world for food, animal feed, decoration, and recreation. The top five pumpkin producing countries are China, India, Russian Federation, the United States (US), and Iran (FAOSTAT, 2011). Approximately 680 million kg of pumpkins are produced in the US every year. Top pumpkin producing states are Illinois, California, New York, Ohio, Pennsylvania, and Michigan (Geisler, 2012). Illinois is the leading state in pumpkin production. Approximately 5,000 ha of jack-o-lantern pumpkin (C. *pepo*) and 5,000 ha of canning pumpkin (*C. moschata*) are produced annually in Illinois. Jack-o-lantern pumpkins are produced for food, animal feed, oil extraction from seed (in Europe), and entertainment (e.g., Halloween celebration in the US). Seeds of some pumpkin cultivars are used as nuts (Wolford and Bank, 2014). Other pumpkin products are pumpkin ice cream, pumpkin wine, pumpkin butter, and pumpkin honey.

Farm-gate value of jack-o-lantern pumpkins in the US ranges from $3,700 to more than $15,000/ha (Babadoost and Zitter, 2009). The canning pumpkin industry is limited to North America. More than 90% of the US processing pumpkins are grown and processed in Illinois (Babadoost and Islam, 2003). Production of processing pumpkins grown in Illinois increased from less than 1,000 ha in 1930s to more than 6,000 ha in 2012. The major processing pumpkin cultivar is Dickinson. The gross value of processing pumpkin products exceeds $ 24,000 per ha (Babadoost and Zitter, 2009).

Common diseases of pumpkin

Pumpkin plants are attacked by several pathogens, including oomycetes, fungi, bacteria, viruses, and nematodes during the growing season. Pumpkin fruit can also be infected after harvest.

Important diseases caused by oomycete are downy mildew (*Pseudoperonospora cubensis*) and Phytophthora blight (*Phytophthora capsici*); major fungal diseases are black rot (*Didymella bryoniae*), Fusarium crown and fruit rot (*Fusarium* spp.), Plectosporium blight (*Plectosporium tabacinum*), powdery mildew *(Erysiphe cichoracearum* and *Sphaerotheca fuliginea)*, and Sclerotinia rot (*Sclerotinia sclerotiorum*); important bacterial diseases include angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*), bacterial fruit blotch (*Acidivorax avenae* subsp. *citrulli*), bacterial spot (*Xanthomonas cucurbitae*), and bacterial wilt (*Erwinia tracheiphila*); common viral diseases are cucumber mosaic [*Cucumber mosaic virus* (CMV)], papaya ringspot [*Papaya ringspot virus* (PRSV)], squash mosaic [*Squash mosaic virus* (SqMV)], watermelon mosaic [*Watermelon mosaic virus* (WMV)], and zucchini yellow mosaic [*Zucchini yellow mosaic virus* (ZYMV)]; and an important nematode disease is southern root-knot (*Meloidogyne incognita*) (Babadoost and Zitter, 2009; Grube, 2011; Jossey and Babadoost, 2008; Langston Jr. et al., 1999; Ravanlou and Babadoost, 2013; Thies et al., 2010; Walcott, 2005).

Bacterial spot

Bacterial spot of cucurbits was first described on 'Hubbard' squash in a New York garden in 1926 (Byran, 1958). The pathogen was identified as *Xanthomonas campestris* pv. *cucurbitae*. Recently, the name of the pathogen was changed to *X. cucurbitae* (Kado, 2010). Since 1926, bacterial spot has been reported from Asia, Australia, Europe, and North America on cucumber, pumpkin, squash, and watermelon (Babadoost and Zitter, 2009; Bineeta et al., 1999; Dutta et al., 2013; Lamichhane et al., 2010; Pruvost et al., 2008). Disease outbreaks have been reported on cucumber, gourd, pumpkin, and summer and winter squash in warm, humid areas (William and

Zitter, 1996). In Illinois, yield losses caused by *X. cucurbitae* exceed 50% in some commercial fields (Babadoost and Zitter, 2009; Babadoost and Ravanlou, 2012; Babadoost et al., 2012).

Foliar symptoms appear as lesions on the leaves and sometimes on young stems and petioles. The lesions on leaves are water soaked, yellow spots, which become brown with age, surrounded by a yellow halo. As lesions coalesce, they may appear more angular and similar to the spots caused by *Pseudomonas syringae* pv. *lachrymans* (Babadoost and Zitter, 2009). The appearance and size of the fruit lesions can vary depending on rind maturity and the presence of moisture. Initial lesions are small (1-3 mm in diameter), slightly sunken, circular spots, with a beige center and a dark brown halo. As the disease progresses, the cuticle and epidermis crack and the lesions enlarge, reaching up to 15 mm in diameter, and become sunken. Penetration of bacteria in the flesh can lead to significant fruit rot in the field or later in the storage (Babadoost and Zitter, 2009).

Taxonomical characteristics of *X. cucurbitae*

The genus *Xanthomonas* belongs to the class Gammaproteobacteria and comprises 27 species that collectively cause serious diseases in about 400 plant hosts (Ryan et al., 2011). *Xanthomonas* bacteria are straight rods (0.4-0.6 × 0.8-2.0 µm), mostly single or in pairs, occasionally short chains, and filaments are rarely seen. Bacteria are obligately aerobic and motile by single polar flagellum. On a sugar containing medium, *Xanthomonas* spp. produce copious exopolysaccharides known as “xanthan gum” and appear as shiny yellow mucoid colonies (except *Xanthomonas axonopodis* pv. *manihotis*) (Kado, 2010). Growth of the bacteria is inhibited by 6% NaCl, 30% glucose, 0.01% lead acetate and methyl green, or by 0.02%

triphenyl tetrazolium chloride (Saddler and Bradbury, 2005). Colonies of all species of *Xanthomonas* are normally smooth, round, entire, and butyrous when young but may show surface markings such as striations and become lobed when older. The production of mucoid, butyrous colonies is an essential feature for distinguishing certain pathogenic *Xanthomonas* spp. from saprophytes (Saddler and Bradbury, 2005).

The taxonomy of the plant pathogenic *Xanthomonas* spp. remains in flux. Since 1980, several studies have been conducted to resolve the taxonomical issues of *Xanthomonas* species. In 1980, five species of *Xanthomonas* including, *X. albinineans*, *X. ampelina*, *X. axonoposis*, *X. campestris*, and *X. fragariae* were listed in the Approved List of Bacterial Names (Skerman et al., 1980). Most of the other previously identified species were placed as pathovar of *X. campestris*. Thus, *X. campestris* had more than 140 pathovars by 1995. Vauterin et al. (1995) carried out a comprehensive DNA-DNA hybridization study by using 183 strains of the genus *Xanthomonas*. The previously described species, *X. campestris* (Pammel 1895) Dowson 1939, was divided into 16 DNA homology groups. Each group was considered as a genomic species (Vauterin et al., 1995).

Two strains of *X. campestris* pv. *cucurbitae (*LMG 690 and LMG 8662) were recovered in DNA hybridization group 8 in the study by Vauterin et al. (1995) that revealed 88% of DNA binding value. The strains were considered sufficiently distinct from all other groups and was described at a species level as *Xanthomonas cucurbitae* (ex Bryan 1926) Vauterin et al., 1995. The G + C content of DNA in type strains ICMP 2299, LMG 690, and NCPPB 2597 was 66.1 to 66.8 mol% (Buttner and Bonas, 2010; Saddler and Bradbury, 2005). *X. cucurbitae* is a yellow, aerobic,

Gram-negative, non-spore forming rod, measuring 0.4-0.6 × 0.5-1.3 µm, bacterium (William and Zitter, 1996). It usually produces only one polar flagellum. On solid culture medium containing utilizable carbohydrates, the colonies are yellow, convex shiny and of a slimy, mucoid.

Epidemiology of bacterial spot disease

Very little is known about the biology of *X. cucurbitae* and epidemiology of bacterial spot. *X. cucurbitae* is reported as a seed-borne pathogen (William and Zitter, 1996). It also survives in plant debris (Babadoost and Zitter, 2009). As infected seed germinates, cotyledons become infected and the pathogen is splashed onto the true leaves. The optimum temperatures for multiplication of *X. cucurbitae* are 25 to 30°C, and the pathogen does not multiply above 35°C (Bineeta et al., 1999). Attempts to isolate the bacterium from soil have been unsuccessful (Babadoost and Zitter, 2009).

Management of bacterial spot

The first step for management of bacterial spot is planting pathogen-free seed (Moffet and Wood, 1979; William and Zitter, 1996). Seed infection may be controlled by soaking the infested seed in a 1:20 dilution of commercial HCl containing 1 % spreader-sticker for 60 minutes. Moffet and Wood (1979) reported that hot water treatments at 54 and 56^{o}C for 30 minutes, and 1% sodium hypochlorite plus 1% spreader-sticker treatment for 40 minutes reduced the level of seed transmission but did not eliminate the pathogen. Also, plantomycin, paushamycin, and streptocycline seed treatments have been reported to reduce the bacterial inoculum in seed. Sinha (1989) reported that cucumber cultivars ‘Japanese Long Green’ and ‘Collection 72-10’ were moderately resistant to bacterial spot. Minimizing moisture on plants by avoiding overhead

irrigation or other means could help to reduce disease development. Crops for both seed and fruit production should be grown in fields that have had no cucurbits for at least 2 years (Babadoost and Zitter, 2009). Weekly copper application from the beginning of fruit set until harvest, forcing the spray into the canopy to cover the foliage and fruit, may reduce incidence of the disease.

RESEARCH OBJECTIVES

Bacterial spot has become a serious threat to pumpkin production in Illinois and other states in the Midwest. It has been reported that some growers ceased pumpkin production because of heavy yield losses to *X. cucurbitae.* No strategies for effective managements of bacterial spot of cucurbits are available, because the host range of *X. cucurbitae* has not been determined, the epidemiology of the disease has not been investigated, and effective chemical and biocontrols for management of the pathogen have not been identified yet. The objectives of this research were: (i) to assess survival of *X. cucurbitae* in plant debris in commercial fields, and (ii) to evaluate efficacy of chemicals and biocontrol agents for control of bacterial spot of pumpkins.

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CHAPTER 2

SURVIVAL OF *XANTHOMONAS CUCURBITAE* IN SOIL

ABSTRACT

This study was conducted to determine the survival of *Xanthomonas cucurbitae*, the causal agent of bacterial spot of cucurbits, in soil. Leaves and fruit infected with *X. cucurbitae* were collected from two commercial fields in Illinois during 25-30 September 2011, and used in this study. A factorial experiment was setup in a secured site at the University of Illinois Fruit and Vegetable Research Farm in Urbana, IL. The experimental factors were: (i) field location where infected leaves and fruit were collected; (ii) plant tissue; (iii) burial depth; and (iv) recovery date. The experimental unit was either five infected leaves or two infected fruit pieces (10 cm × 10 cm), which were placed in fiber glass mesh and buried either at 0-10 cm or 10-20 cm deep on 7 October 2011. During the season, the experimental site was kept clean of plants by hand-weeding. Temperature at the site was recorded at 1 m above the ground, at the soil surface, and at 5 and 15 cm depths. Also, soil moisture at 5 and 15 cm depths were recorded. The samples were recovered on 7 April, 7 July, and 7 October 2012; and 7 January, 7 April, and 7 October 2013. Recovered samples were tested for presence of *X. cucurbitae.* Each sample was processed in phosphate buffered saline and sterile distilled water. Dilutions of 10^{0}, 10^{-1}, 10^{-2}, and 10^{-3} were prepared from ground sample, and 100 µl of each dilution was spread on kasumagycin-cephalexin agar (KC) medium in Petri dishes. *Xanthomonas*-like colonies were sub-cultured on yeast dextrose agar (YDC) medium. Single-cell colonies were obtained and were identified. Identification of *X. cucurbitae* isolates was based on the colony characteristics on YDC and pathogenicity tests. Mean colony forming unit (CFU) values of *X. cucurbitae* per g of dry plant

tissue were calculated. The location of the field did not significantly (P = 0.7895) affect the number of *X. cucurbitae* CFUs recovered from the samples. However, survival of *X. cucurbitae* was significantly affected by burial depths (P = 0.08), the plant tissue (P = 0.001) and time period of plant tissue in soil (P = 0001). The interaction of the plant tissue and the time period of plant tissue in soil was also significant (P = 0.0018) on survival of *X. cucurbitae,* but interactions of other factors on the survival of *X. cucurbitae* were not significant.

INTRODUCTION

Xanthomonas spp. are foliar pathogens, causing blight, spot, and blotch on leaves and stems, and a few of them cause cankers and the gumming of inflorescences (Kado, 2010). The bacteria survive in the seeds and in perennial hosts. Although some *Xanthomonas* spp. overwinter in infected plant debris in soil, they do not multiply or survive in the free-state in soil, and none of them can be characterized as soil inhabitant (Scroth, 1979). However, *X. alfalfa* has been reported to overwinter in soil apparently free of plant debris (Claflin and Stuteville, 1973).

It has been shown experimentally that the following bacteria declined rapidly and reached extinction within days or weeks after introducing them in to soil: *X. pelargoni, X. vasculorum, X. phaseoli, X. translucens, X. malvacearum, X. citri, X. campestris, X. vesicatoria, and X. oryzae* (Scroth, 1979). However, Claflin and Stuteville (1973) reported that when infected debris was stored dry, *Xanthomonas* spp. survived from 8 months to 8 years. Milus and Mirlohi (1995) reported that *X. campestris* pv. *transulens* was not detected in crop debris, soil, or possible alternative host plants in the field 3 months after harvest. *X. campestris* pv. *pelargonii* survived up to 221 days in air-dried leaves placed on the surface of a greenhouse bench but was not detected after 11 days in diseased leaf buried in soil (Kennedy et al.,1987). *X. axonopodis* pv.

phaseoli var. *fuscans (Xap)* survived for 65 to 180 days in the leaflets on the soil surface, and for 30 to 120 days in those incorporated into the soil depths of 10 and 15 cm. When higher rainfall and temperatures occurred *Xap* survival was from 45 to 60 days in leaflets on the soil surface and from 30 to 45 days in those buried in soil (Torres et al., 2009). *X. oryzae* overwintered on unhulled rice grains (Wakimoto, 1955). There is considerable variation in the reported survival times of *Xanthomonas* spp. in seeds. For example, *X. phaseoli* has been reported to survive for three (Basu and Wallen, 1966) and 15 years (Schuster and Coyne, 1974).

The ability of *Xanthomonas* spp. to survive in plant debris may enable the bacterium to overwinter under certain conditions. Since the population becomes undetectable soon after infested debris is decomposed, survival is dependent upon the rate of decay of infested plant tissues and would vary markedly depending on the environmental conditions (Scroth, 1979).
There have been no studies on survival of *X. cucurbitae* in soil. The objective of this study was to assess the survival of *X. cucurbitae* in plant debris in soil.

MATERIALS AND METHODS

Survival of *X. cucurbitae* in the field. An experiment was conducted at the University of Illinois Fruit and Vegetable Research Farm, Urbana, IL (latitude 40^{o}04’.7”N; longitude 88^{o}12’.9”W; elevation 236 m) to assess survival of *X. cucurbitae* in plant tissues in the field. The soil texture was silt loam with pH 6.7 and 4.5% organic matter. The field had been planted to soybean prior to setting up the experiment. An area of 20 m × 15 m was plowed and disked on 18 September 2011 to prepare for the experiment.

Infected leaves and fruit with *X. cucurbitae* were collected from two commercial fields; one field in Putnam County (designated as northern field) and another field in Champaign County (designated as central field). All samples were from pumpkin 'Howden'. The infected leaves and fruit samples were collected during 25-30 September 2011 and were kept at 4^{o}C until preparing experimental samples. Each experimental sample was either five infected leaves (about 120 g) or two infected fruit pieces (10 cm × 10 cm each). Samples were prepared during 5-6 October. Asymptomatic leaves from pumpkin 'Howden' plants grown in a greenhouse were included as control check. Each leaf or fruit sample was placed in a fiber glass mesh bag (35 cm × 35 cm) and the bags were tied with non-biodegradable nylon string. The samples were kept at 4^{o}C until placed in the experimental site in the field on 7 October 2011.

The experiment was performed in a factorial design with four replications. The factors were: (i) location of the fields where plant samples were collected from (northern and central fields); (ii) plant tissue (leaf and fruit); (iii) burial depth of tissue (0-10 and 10-20 cm); and (iv) recovery date (time) of the samples. Temperature at the experimental site was recorded hourly at 1 m above the ground, at the soil surface, and at 5 and 15 cm depths using a WatchDog datalogger (model 400, Spectrum Technologies, Inc., Plainfield, IL). Also soil moisture was recorded hourly at 5 and 15 cm depths using a WatchDog datalogger (model 1000, Spectrum Technologies, Inc., Plainfield, IL). During the growing seasons the experimental site was kept clean of plants by hand-weeding.

Isolation of *X. cucurbitae* from samples. Initial isolation of *X. cucurbitae* from leaf and fruit samples was made 48 hr prior to burying samples. Surface of leaf or fruit tissues was disinfested

by gentle rubbing with blotters soaked in 99% ethanol and the area (pieces 2- to 4-mm diameter) with lesions was excised using a sterile scalpel in a laminar flow hood. Each piece of leaf or fruit tissue with lesions was immersed into a 1.5 ml microcentrifuge tube containing 1 ml sterilized distilled water (SDW). The tube was shaken using a shaker (Thermolyn Maxi Mix ®II, Barnstead International, IA) for 30 sec, and then a loopful of the suspension was streaked onto kasugamycin-cephalexin agar (KC) in Petri dishes, as described by Pruvost et al. (2010). KC medium contained 7 g yeast extract, 7 g peptone, 7 g glucose, 18 g agar, 20 mg propiconazol, 40 mg cephalexin, and 20 mg kasugamycin in 1,000 ml distilled water. KC is a semi-selective medium for *Xanthomonas* spp. (Schaad et al., 2001; Swamy, 2008).

Buried samples were recovered on 7 April, 7 July, and 7 October 2012; and 7 January, 7 April, and 7 October 2013. At each recovery time, 40 sample bags were removed and processed. The processed bags included four control leaf samples buried at 0-10 cm, four control leaf samples buried at 10-20 cm, four leaf samples from northern field buried at 0-10 cm, four leaf samples from northern field buried at 10-20 cm; four fruit samples from northern field buried at 0-10 cm, four fruit samples from northern field buried at 10-20 cm; four leaf samples from central field buried at 0-10 cm, four leaf samples from central field buried at 10-20 cm; four fruit samples from central field buried at 0-10 cm, and four fruit samples from central field buried at 10-20 cm. A suspension of each sample was prepared in phosphate buffered saline (PBS). PBS buffer contained 3.9 g KH_2PO_4, 2 g KCl, 80 g NaCl, and 17.9 g Na_2HPO_4 in 1,000 ml distilled water with pH 7.1. Dilutions of 10^0, 10^{-1}, 10^{-2}, and 10^{-3} of the suspension were prepared. Similar dilutions were also made in SDW. One g of the processed tissue was added to 9 ml of PBS or SDW in a 30-ml glass tube, which was considered as the 10^0 dilution. The tube was shaken by

hand for 5 sec. Then, 1 ml suspension was transferred to a new tube with 9 ml of PBS or SDW to make 10^{-1} dilution. Similarly, 10^{-2} and 10^{-3} dilutions were prepared.

Aliquots of 100 µl of each diluted suspension in PBS or SDW were added onto the surface of the KC medium in Petri dishes. The plates were incubated in the dark at 28 ± 1°C. After 4 days, *Xanthomons*-like yellow colonies were sub-cultured by streaking onto yeast extract dextrose calcium carbonate (YDC) medium (Schaad et al., 2001). YDC medium contained 10 g yeast extract, 20 g dextrose, 20 g calcium carbonate, and 15 g agar in 1,000 ml distilled water. The YDC culture plates were incubated in the dark at 28 ± 1°C (Mohammadi et al., 2001; Schaad et al., 2001). Developing colonies were sub-cultured twice on Luria Bertani agar (LB) medium to produce single cell colonies. After 2 days, single-cell colonies were transferred into 1.2 ml cryogenic vials (Corning Incorporated, Life Sciences, Acton, MA) containing 1 ml solution of 15 or 30% glycerol for storage at -20°C and -80°C, respectively (Goto et al., 1980; Schaad et al., 2001) for the future studies.

Colony per g of plant tissue. During the above-described isolation process the number of *X. cucurbitae* colonies was calculated. Four days after culturing on KC medium in Petri dishes, the number of colony forming unit (CFU) of *X. cucurbitae* was counted and CFU per g of plant debris was calculated.

Identification of *X. cucurbitae*. *X. cucurbitae* was identified based on the following tests.

Cultural test. Morphological characteristics of the bacterial colonies on YDC was the first step for consideration of the colonies as *Xanthomonas*. Colonies of *Xanthomonas* spp. are mucoid, convex, yellow on YDC (Schaad et al., 2001).

Pathogenicity of Xanthomonas isolates. The isolates with mucoid, convex, yellow colonies on YDC were tested for their pathogenicity on pumpkin 'Howden' in a greenhouse. Howden is a susceptible pumpkin cultivar to *X. cucurbitae.* From each recovery date, 22-28 *Xanthomonas* isolates were tested for their pathogenicity. Each isolate was cultured on LB agar at 24 ± 1°C for 3 days. Bacterial inoculum was prepared by washing bacterial colonies with SDW into a 15 ml conical tube (Falcon 352097). The density of bacterial cells was adjusted to 5×10^7 CFU/ml using a spectrophotometer (Smart Spec 3000; Bio-Rad, Philadelphia, PA) at OD600 nm (OD = 0.5 at 600 nm). Three-week-old pumpkin plants were inoculated by infiltration of the bacterial suspension into the abaxial side of leaves, using a 10 ml syringe. ATCC strain 23378 of *X. cucurbitae* was used as positive control and SDW as negative control. For each isolate two leaves from the same plant were inoculated by three infiltrations per leaf. Inoculated plants were placed in a greenhouse maintained at 26 ± 2°C. Development of the disease symptoms (water-soaked, necrotic lesions) were recorded every day from 5 to 10 days post inoculation. Symptomatic tissues were processed to isolate *X. cucurbitae.* Isolated bacterial colonies were identified based on the colony characteristics on YDC (Barak et al., 2001). The experiment for each isolate was repeated once.

Hypersensitivity reaction test. Hypersensitivity reaction test was conducted on young fully developed leaves of tobacco (*Nicotiana benthamiana*) by testing five isolates from each recovery

date (a total of 30 isolates). Tobacco leaves were inoculated with an *X. cucurbitae* suspension (10^8 CFU/ml). Using a 10-ml syringe, 0.5 ml of the bacterial suspension was infiltrated into interveinal leaf tissue on the abaxial side. ATCC strain 23378 of *X. cucurbitae* and SDW were used as positive and negative controls, respectively. Inoculated plants were placed in a greenhouse maintained at 26 ± 2°C. The plants were checked for development of hypersensitive reaction within 24-48 hr after injection (Kado, 2010; Kim et al., 2003; Vanneste et al., 1990). Each isolate was tested twice, with three replications.

Data analysis. The data were analyzed in SAS (version 9.3, SAS Inc., Cary, NC). Data of the colony forming unit were log transformed to meet the valid assumptions of normality and variance. Analysis of variance (ANOVA) was conducted using PROC GLM to determine the effects of the fixed four factors (field location, plant tissue, burial depth and time). The significance of effects of factors were determined at $\alpha = 0.1$. Least significant different (LSD) at $\alpha = 0.05$ was calculated to compare differences among average number of CFU of different plant parts over time. A regression analysis in excel was performed to predict survival of *X. cucurbitae* in soil over time (cross location, depth, and plant tissue) using the mathematical equation $y = a + bx$, in which y = predicted $\log_{10}$ number of CFU, a = number of CFU at 6-month recovery, and b = rate of decline of CFU over time, and x = time (month). Similarly, regression analyses were performed for survival of *X. cucurbitae* in leaf and fruit tissues separately and for burial depths.

RESULTS

Isolation of *X. cucurbitae* from samples. *X. cucurbitae* was isolated from both originally infected leaf and fruit samples 6, 9, 15, 18, and 24 months from the time they were placed in the

soil. Colonies of the isolated bacteria were mucoid, convex yellow on YDC. None of the bacterial isolates from originally asymptomatic leaf samples produced mucoid, yellow colonies on YDC.

Plants with SDW injection did not produce water-soaked lesion (Figure 2.1). But, plants inoculated with all of the isolates with mucoid, yellow colonies on YDC caused water-soaked brown lesions with a yellow halo (Figure 2.2) on pumpkin 'Howden', like the lesions produced by the ATCC strain 23378 (Figure 2.3). The bacterium was re-isolated from the inoculated plants, and the isolated bacteria produced mucoid, yellow colonies on YDC. Based on the characteristic of the isolates on YDC, and development of water-soaked brown lesions on inoculated pumpkin plants, the isolated bacteria were identified as *X. cucurbitae*.

Colony per g of plant tissue. There was no significant effect of samples collected from different field location (P = 0.79) in the survival of *X. cucurbitae* (Table 2.1). But survival of *X. cucurbitae* in soil was significantly affected by burial depth of the samples (P = 0.08), plant tissue (P = 0.0001), and time period of plant tissue in soil (P = 0.0001). The interaction of the plant tissue and the time period of plant tissue in soil on the survival of *X. cucurbitae* was significant (P = 0.0018) (Table 2.1).

There were significant differences in the number of *X. cucurbitae* colonies per g tissue recovered from plant samples at different recovery dates (months) (Figure 2.4). Recovered number of CFU after 6 months (April 2012) was significantly greater than those recovered after 9 months (July 2012). Similarly, number of CFU recovered after 9 months (July 2012) was significantly greater

than those recovered after 12 months (October 2012). There was no significant difference between numbers of CFU recovered after 12 months (October 2012) and 18 months (April 2013), but numbers of CFU recovered after 12 months (October 2012) and 18 months (April 2013) were significantly greater than those recovered after 15 months (January 2013) and 24 months (October 2013). Number of CFU recovered after 24 months (October 2013) was significantly fewer than those recovered before 18 month (April 2013) (Figure 2.4). The biggest decline in number of CFU/g plant tissue was from 6 months (October 2011) to 9 months (July 2012) after placing samples in the soil.

The number of CFU recovered from fruit samples were significantly higher (P = 0.05) at 6 months (April 2012), 9 months (July 2012), and 18 months (April 2013) recoveries, compared to the number of CFU recovered from leaf samples (Figure 2.4). The number of CFU recovered from fruit and leaf samples at 12 months (October 2012), 15 months (January 2013), and 24 months (October 2013) were not significantly different from each other (Figure 2.4).

The relationships between CFU recovered from the samples to the time period (month) of samples in the soil were linear. The overall relationship between $\log_{10}$ of CFU number and the time period (month) was $y = 4.8779 - 0.0994x$ ($R^2 = 0.67$), in which $y = \log_{10}$ of CFU number and x = time period (month) (Figure 2.5). Similarly, the relationships between $\log_{10}$ of CFU number and time period (month) were: for leaf samples, $y = 4.6444 - 0.0899x$ ($R^2 = 0.63$) (Figure 2.6); fruit samples, $y = 5.1286 - 0.1099x$ ($R^2 = 0.74$) (Figure 2.6); sample burring depth of 5 cm, $y = 4.9108 - 0.1036x$ (Figure 2.7) ($R^2 = 0.72$); and sample burial depth of 15 cm, $y = 4.8622 - 0.0926x$ ($R^2 = 0.62$) (Figure 2.7).

The lowest daily mean temperature at the 5 cm depth was -1.21^{o}C and at the 15 cm depth was 0.56^{o}C during October-December 2011 (Figure 2.8). From December of 2011 to February of 2012, the lowest temperature at the 5 and 15 cm depths were -3 and 1^{o}C, respectively. During June-July of 2012, temperature at both depths increased rapidly and reached 38^{o}C at the 5 cm depth and 29^{o}C at 15 cm depth. The lowest daily mean temperature recorded during January-February of 2013 was -4^{o}C at the 5 cm depth and -2.5^{o}C at the 15 cm depth. During June-July of 2013, temperatures at both depths increased rapidly and reached 32 and 25^{o}C at 5 and 15 cm depth, respectively (Figure 2.8).

In 2012, the daily mean moisture recorded at 5 cm depth had more fluctuation than that of 15 cm depth (Figure 2.9). Recorded low moistures of soil in 2012 for 5 and 15 cm were during April-May and September-October, respectively. In 2013, the daily mean moisture recorded at 5 cm depth had more fluctuation than 15 cm depth. The daily mean moisture at 5 cm depth was lower than that of 15 cm depth during February-May, moisture at 5 cm depth was greater than that of 15 cm depth during May-June, and moisture levels at both 5 and 15 cm depths were almost the same during August-September 2013 (Figure 2.9).

DISCUSSION

Survival of *Xanthomonas* species in soil have been studied by several researchers who have reported relatively rapid decline of the bacteria in the field (Bashan, 1982; Scroth, 1979; Torres et al. 2009). Torres et al. (2009) reported that *X. axonopodis* pv. *phaseoli* var. *fuscans (Xap)* survived up to 180 days in leaves placed on the soil surface, but up to 120 days in leaves buried in the soil to depths of 10 and 15 cm. Claflin and Stuteville (1973) reported survival of

Xanthomonas spp. in infested debris from 8 months to 8 years. After studying the survival of *X. campestris pv. vesicatoria*, Bashan (1982) reported that only a few CFU were isolated from pepper debris after 18 months from the time the debris left in the soil. The result of our research showed that *X. cucurbitae* survived 24 months in infected leaf and fruit tissues of pumpkin in the soil. We have an ongoing study for determining the exact period of time that *X. cucurbitae* would survive in soil. The predicted zero CFU value in the linear regression models are as follows: for leaf tissues: 52 months; fruit tissues: 47 months; leaf and fruit tissues combined at 5 cm depth: 47 months; and leaf and fruit tissues combined at 15 cm depth: 50 months.

The possible reasons for relatively longer survival period of *X. cucurbitae* in the soil, compared to survival of other *Xanthomonas* species could be: (i) *X. cucurbitae* may survive longer in the plant tissue in the soil than other *Xanthomonas* spp.; (ii) the decomposition of pumpkin leaf and fruit tissues takes a longer time, thus these tissues support a longer period of survival of *X. cucurbitae*; and (iii) *Xanthomonas* spp. survival may differ in different soil textures. Additional research is needed to determine the survival of *X. cucurbitae* in different host tissues, in different soil textures, and under different climatic conditions.

Infected leaf and fruit samples from two fields (northern and central fields) were included in this study. The reasons for including samples from two different fields were: (i) detected genetic variation between *X. cucurbitae* isolates from the fields in a different study; and (ii) possibility of differences in decomposing plant tissues from different fields. The results, however, showed that there was no significant difference ($P = 0.79$) between CFU numbers recovered from samples

from the fields. The samples from both fields were from pumpkin ‘Howden’. Samples from other pumpkin cultivars may show different results.

Based on the regression analysis, *X. cucurbitae* survives a relatively shorter period of time at the shallow depth (0-10 cm deep) than the deeper level (10-20 cm) in soil. A possible reason for this result could be that there was more fluctuation of temperature and moisture, and higher oxygen level in shallow depth than in deeper level. But in both cases, *X. cucurbitae* is predicted to survive for 47 months or longer in soil. One possibility for reducing time period for survival of *X. cucurbitae* in soil could be enhancing the decomposition of pumpkin plant debris after harvest. Additional research is needed to determine the time needed by pumpkin plants to decompose in soil.

Even a small number of surviving *X. cucurbitae* cells in plant debris may results in an outbreak of bacterial spot, as cells of *X. cucurbitae* rapidly multiply and produce high populations, as reported by Schuster and Coyne (1974). Currently, a two-year crop rotation with non-host plants is recommended for the management of bacterial spot of pumpkin (Babadoost and Zitter, 2009). Although, we do not have sufficient information yet to declare the exact time period for survival of *X. cucurbitae* in soil, the results of ours research show that a two-year crop rotation, which is currently recommended for management of bacterial spot of cucurbits, is not sufficient for effective management of *X. cucurbitae* in pumpkin.

The recovered number of *X. cucurbitae* CFU per g tissue of plant samples showed a decreasing pattern of survival of the bacterium over time. However, the number of CFU recovered after 18

months (April 2013) was greater than number of CFU after 15 months (January 2013). This could be due to multiplication of *X. cucurbitae* cells during the warmer conditions of March and April 2013. According to Bineeta et al. (1999), *X. cucurbitae* does not multiply below 5^{o}C. Daily mean temperature measured in the soil at the experimental site ranged from -3^{o}C to 4^{o}C during January 2013, whereas temperature ranged from -0.2^{o}C to 20^{o}C during April 2013.

TABLES AND FIGURES

Table 2.1. Analysis of variance of effects of field location, plant tissue, burial depth of tissue, and time period on survival of *Xanthomonas cucurbitae* in soil[v].

Source	**F value**	**Pr > F**
Field location[w]	0.07	0.7895
Plant tissue[x]	26.38	0.00001
Depth[y]	3.13	0.0807
Recovery date (time)[z]	200.83	0.0001
Plant tissue*time	4.23	0.0018

[v] The experiment was conducted at the University of Illinois Fruit and Vegetable Research Farm in Urbana, IL during 2011-2013.

[w] Two fields from northern and central Illinois, where infected leaves and fruit were collected from for the experiment.

[x] Leaf and fruit samples.

[y] Burial depths of samples tissues were 0-10 and 10-20 cm.

[z] Recovery date (6, 9, 12, 15, 18, and 24 months) from the date of placing samples in the soil.

Figure 2.1. A pumpkin ‘Howden’ leaf 7 days after infiltrated with sterile distilled water.

Figure 2.2. Lesions on pumpkin ‘Howden’ leaves 7 days after inoculation with a *Xanthomonas cucurbitae* isolated from infected pumpkin leaves buried 10-20 cm deep in the field for 24 months.

Figure 2.3. Lesions on pumpkin ‘Howden’ leaves 7 days after inoculation with the ATCC strain 23378 of *Xanthomonas cucurbitae.*

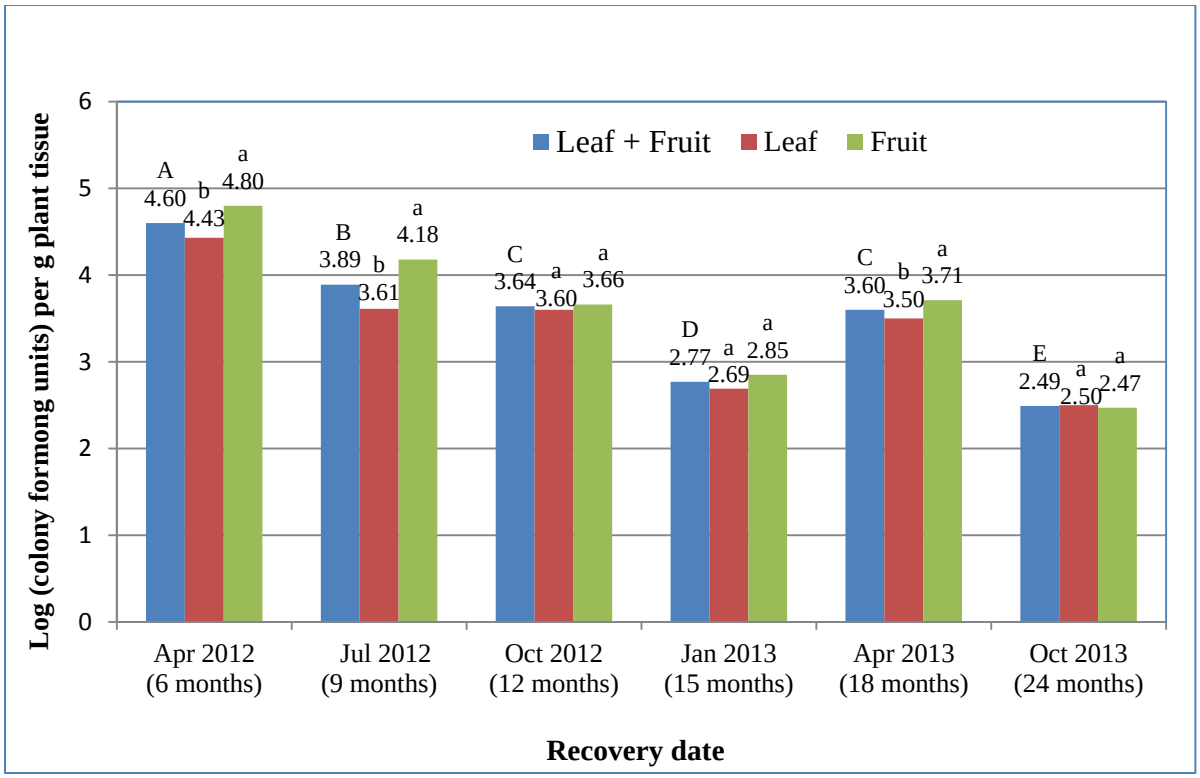


Figure 2.4. Survival of *Xanthomonas cucurbitae* in infected leaves and fruit of pumpkin in soil at the University of Illinois Fruit and Vegetable Research Farm in Urbana, IL during 2011-2013. Colony forming unit (CFU) of *X. cucurbitae* was determined by culturing plant tissues on kasugamycin-cephalexin agar. Bars with average $\log_{10}$ CFU of leaves and fruit with a letter in common are not significantly different from each other according to Fisher's protected LSD (P = 0.05). At each recovery date, bars with CFU numbers of leaves and fruit with the same letter are not significantly different from each other at P = 0.05.

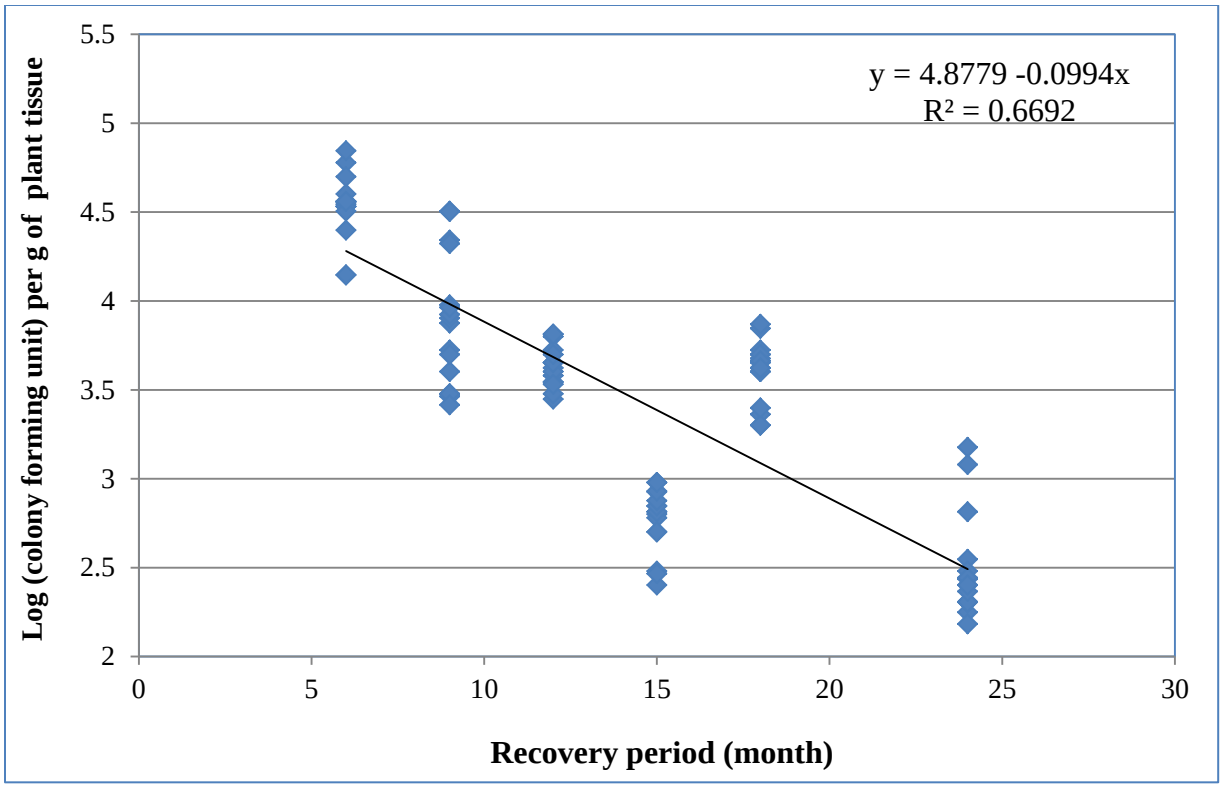


Figure 2.5. Regression of $\log_{10}$ colony forming unit (CFU) of *Xanthomonas cucurbitae* recovered from infected leaf and fruit samples of pumpkin buried in soil for a period of 24 months during October 2011 - September 2013 at the University of Illinois Fruit and Vegetable Research Farm in Urbana, IL. CFU number for each recovery period represents an average of 32 samples (16 leaf and 16 fruit samples) collected from two different fields and buried at 0-10 and 10-20 cm depths.

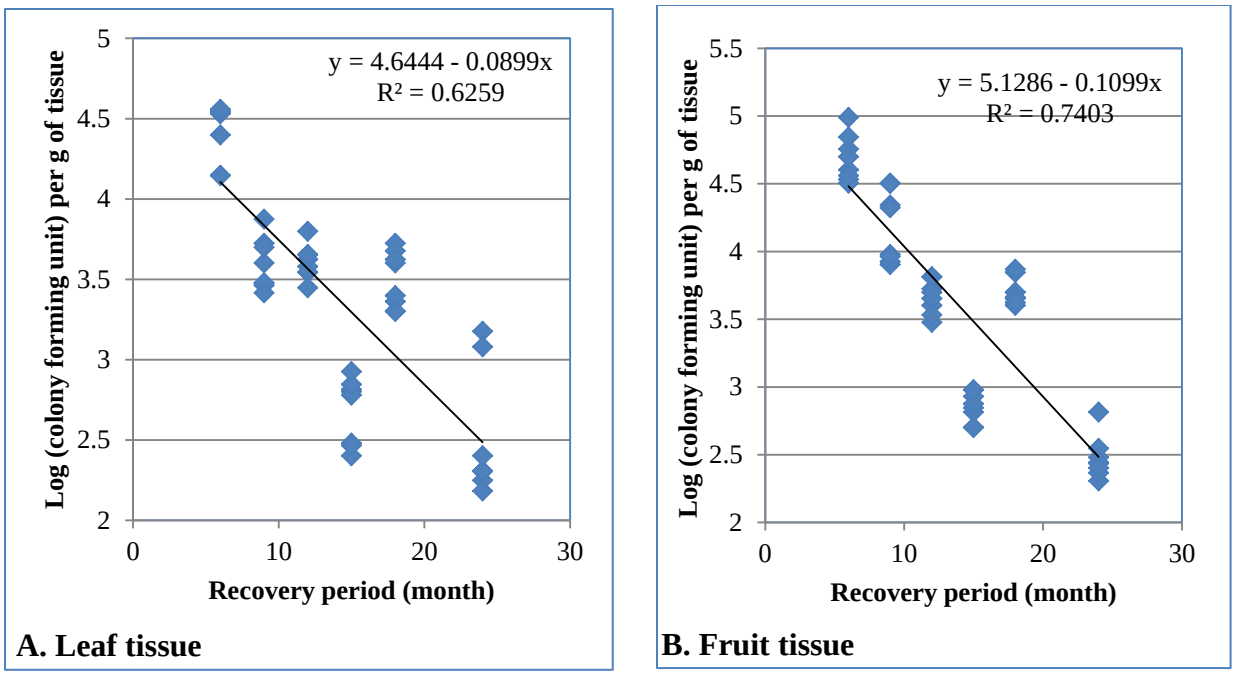


Figure 2.6. Regression of $\log_{10}$ colony forming unit (CFU) of *Xanthomonas cucurbitae* recovered from either infected leaf or infected fruit samples of pumpkin buried in soil for a period of 24 months during October 2011 - September 2013 at the University of Illinois Fruit and Vegetable Research Farm in Urbana, IL. In each of the graphs, CFU number for each recovery period represents an average of 16 samples collected from two different fields and buried at 0-10 and 10-20 cm depths.

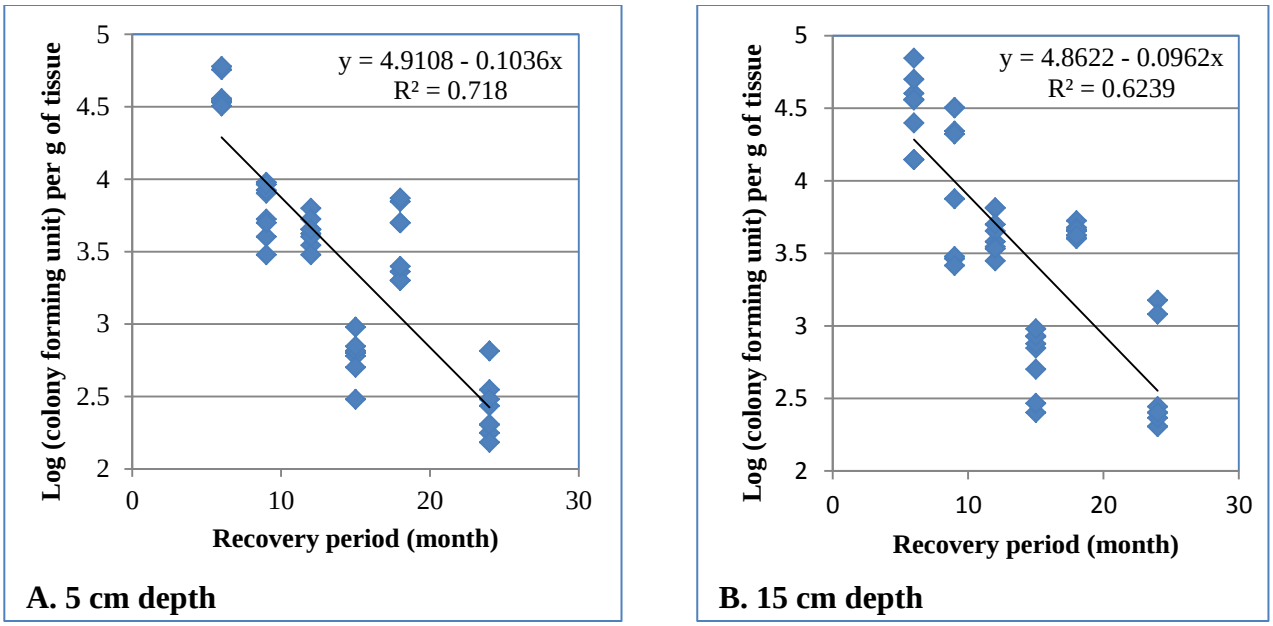


Figure 2.7. Regression of $\log_{10}$ colony forming unit (CFU) of *Xanthomonas cucurbitae* recovered from infected leaves and fruit samples of pumpkin buried at either 0-10 (5) cm or 10-20 (15) cm for a period of 24 months during October 2011 - September 2013 in the fields at the University of Illinois Fruit and Vegetable Research Farm in Urbana, IL. In each of the graphs, CFU number for each recovery period represents an average of 16 samples (eight leaf and eight fruit samples) collected from two different fields.

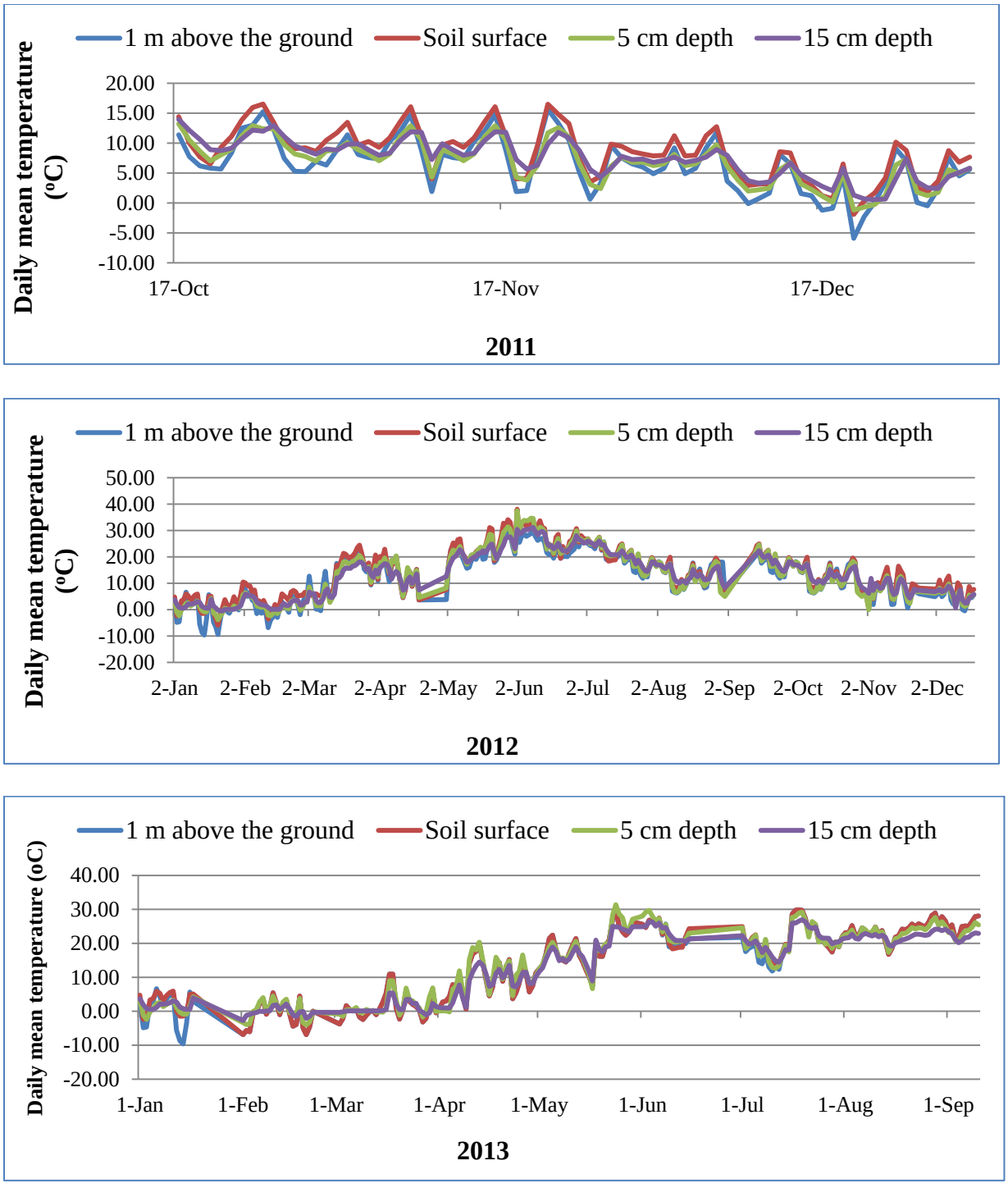
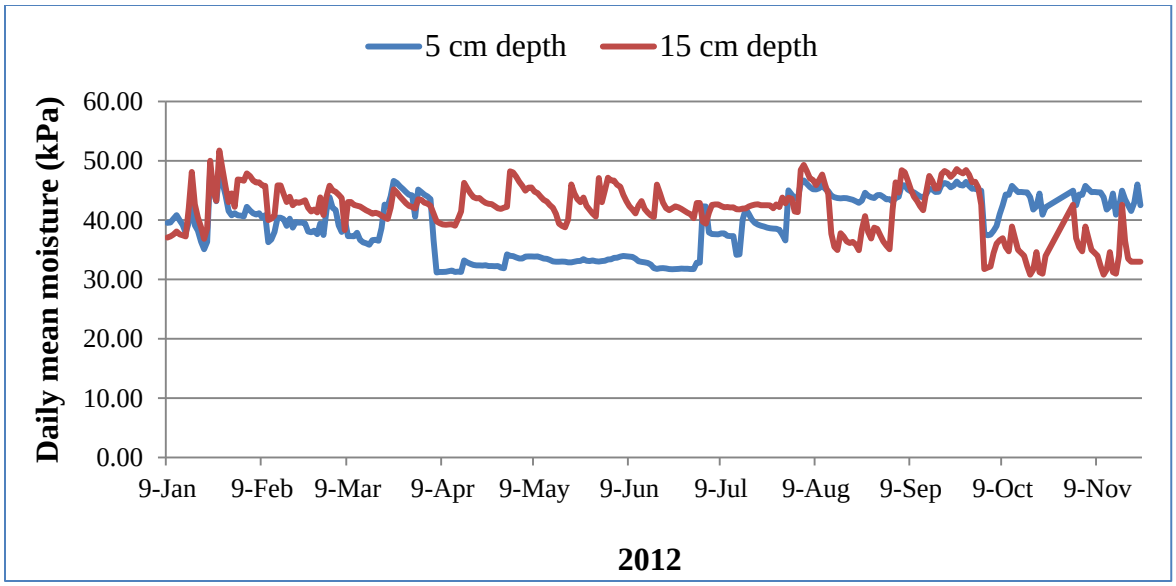


Figure 2.8. Daily mean temperature (°C) at 1 m above the ground, soil surface, and 5 and 15 cm depths at the experimental site at the University of Illinois Fruit and Vegetable Research Farm, Urbana, IL, from October 2011 to September 2013.



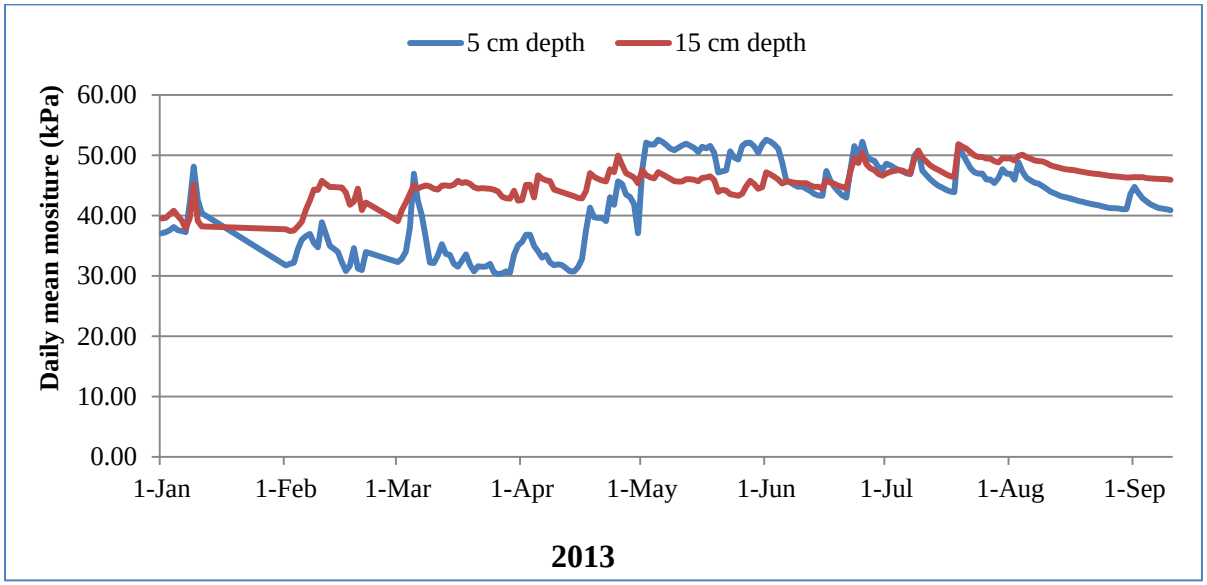


Figure 2.9. Daily mean moisture at 5 and 15 cm depths at the experimental site at the University of Illinois Fruit and Vegetable Research Farm, Urbana, IL, from January 2012 to September 2013.

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CHAPTER 3

EFFICACY OF CHEMICALS AND BIOCONTROL AGENTS FOR CONTROL OF BACTERIAL SPOT OF PUMPKIN, CAUSED BY *XANTHOMONAS CUCURBITAE*

ABSTRACT

This study was conducted to evaluate the efficacy of selected chemicals and biocontrol agents for control of bacterial spot, caused by *Xanthomonas cucurbitae* of pumpkin. The chemicals were acibenzolar-s-methyl (ActiGard 50 WG); copper compounds (Agion E, Badge X_2 DF, Cueva FL, Cuprofix Ultra 40 DF, Kocide-3000 46.1 DF, Nordox 75 WG, and Phyton-016 B), copper + zinc + silver salts (SciEx83-3S, and SciEX83-4S), famoxadone + cymoxanil (Tanos 50 DWG), kasugamycin (Kasumin 2L), mancozeb (Dithane 75 DF), oxytetracycline (Mycoshield 40 WSP), potassium carbonate (Milstop SP), quinolin (Quintec 2.08 SC), and streptomycin (AgriMycin 17 WP). The biocontrols were *Bacillus amyloliquefaciens* (Cx-9030 WDG), *B. subtilis* (Serenade ASO), *B. pumilus* (Sonata ASO), *Streptomyces lydicus* (Actinovate AG), and an extract of *Reynoutria sachalinensis* (Regalia). Laboratory studies of the chemicals were conducted to determine the effective concentration for 50% and 100% reduction in cell multiplication (EC_{50} and EC_{100}) values. Ten isolates of *X. cucurbitae* collected from different parts of Illinois were tested in nutrient broth (NB) and casitone yeast extract broth (CYE). There was a significant difference between EC_{50} values in the two culture media. The EC_{50} values of the chemicals ranged from 0.17 ppm mancozeb (Dithane) to 64.53 ppm cuprous oxide (Nordox) in NB, and from 0.23 ppm mancozeb (Dithane) to 38.87 ppm cuprous oxide (ppm Nordox) in CYE. Similarly, the EC_{100} values of the chemicals ranged from 1 ppm mancozeb (Dithane) to 175 ppm cuprous oxide (Nordox) in NB, and from 1 ppm mancozeb (Dithane) to 125 ppm cuprous oxide

(Nordox) in CYE. The Laboratory tests of biocontrol agents were conducted using the filter-disc assay method. All tested biocontrol agents were effective in preventing multiplication of *X. cucurbitae* cells around the filter disc. The field trials were carried out at the University of Illinois Vegetable Research Farm in Champaign, IL. Each experiment was performed in a randomized complete block design with three replications. Jack-o-lantern pumpkin 'Howden' was used in this study. Plants were inoculated with the *X. cucurbitae* suspension (5×10^7 CFU/ml) using a backpack sprayer. Spray-application of chemicals began either pre- or post-inoculation of plants. Spray-application of all biocontrol agents began pre-inoculation. All chemical and biocontrol compounds were applied at 7-day intervals. Incidence and severity of bacterial spot in leaves were significantly lower in treated plots than those of untreated control plots. Treatments with Badge, Cuprofix, Mycoshield, Phyton, Kocide plus ActiGard, and Kocide plus Tanos were more effective in reducing incidence and severity of bacterial spot on both leaves and fruit than other chemical treatments. Biocontrols Regalia and Serenade were more effective in reducing incidence and severity of bacterial spot on both leaves and fruit.

INTRODUCTION

Bacterial spot, caused by *Xanthomonas cucurbitae*, has become a serious threat to pumpkin producers in Illinois, as well as in other states in the Midwest. Surveys in the past four years showed that the disease occurred in more than 80% of pumpkin fields in Illinois, causing up to 90% yield losses in some fields (Babadoost and Ravanlou, unpublished data). No effective strategy for management of bacterial spot of cucurbits is available.

Thirumalahar et al. (1956) reported that chloramphenicol, streptomycin sulphate, terramycin, and aureomycin inhibited colony development of *X. phaseoli* pv. *sojense*. Mahir et al. (2005) tested chemicals (streptomycin sulphate, Dithane M-45, Agrimycin-100, Vitavax, Benlate, and Cobox) at 1% concentration against multiplication of *X. campestris* pv. *citri in-vitro*. They reported that Agrimycin-100, streptomycin sulphate, Vitavax, and Dithane M-45 were more effective at reducing cell multiplication than other chemicals. Agrimycin-100, streptomycin sulphate, DithaneM-45, and Vitavax were further studied *in vitro* against colony formation of *X. campestris* pv. *citri* at 0.01, 0.1, and 1% concentrations. All four chemicals inhibited the multiplication of the bacteria at all concentrations; however, streptomycin sulphate was found to be the most effective. The antagonism of eight *Bacillus* isolates was investigated against nine isolates of *X. campestris* pv. *campestris* (causal agent of crucifers black rot), and it was reported that four of the *Bacillus* isolates (R14, RAB7, C116, and C210) produced inhibition zones (Monteiro, et al., 2005)

Dhakal et al. (2009) reported that spray applications of different chemicals, including copper oxychloride (2.5%) and Bordeux mixture (1 and 2%), decrease the severity of citrus canker caused by *X. citri.* In the field trial, kasugamycin (Kasumin 2L) reduced the severity of bacterial spot of tomato (*X. perforans*) as effective as the standard copper plus mancozeb treatment (Vallad et al., 2010). In a study conducted by Roberts et al. (2008), famoxadane + cymoxanil (Tanos 50 DWG) was reported to be effective in suppressing bacterial spot of tomato (*X. perforans*). Roberts et al., (2008) reported that acibenzolar-s-methyl (ActiGard 50 WG), a systemic acquired resistance inducer, reduced the severity of bacterial spot of tomato; however, the reduction level was the same as that resulting from the treatment with copper (Kocide-2000

DF) plus mancozeb (Manzate 75 DF) sprays. In the same study, spray-applications of *B. subtilis*, strain QST 713 (Serenade WPO) plus copper hydroxide (Kocide-2000 DF) significantly reduced the severity of bacterial spot of tomato as compared to untreated plants.

X. campestris pv. *vesicatoria*, the causal agent of bacterial spot of pepper, was tested for copper and streptomycin resistance in Turkey. All 67 isolates tested were found to be tolerant to 100 µg/ml of copper (cupric sulfate, Merck) and 7% were resistant to 20 µg/ml of streptomycin (streptomycin sulpate, Sigma) (Miriki et al., 2007). Ritchie and Dittapongpitch (1991) reported that there are copper and streptomycin resistant strains of *X. campestris* pv. *vesicatoria*. In surveys of 32 noncontiguous fields during four growing season, 63% of 70 isolates were found to be copper resistant and 30% were resistant to at least 100 µg/ml of streptomycin. All streptomycin resistant isolates were also resistant to copper. Behlau et al., (2011) reported that long-term use of copper-based bactericides has led to the development of copper resistant strains in both *X. citri* subsp. *citri* and *X. alfalfa* subsp. *citumelonis* in citrus.

There are no reports on the sensitivity or resistance of *X. cucurbitae* to chemicals and biocontrols available. The objective of this study was to evaluate the efficacy of some selected chemicals and biocontrols against *X. cucurbitae* in the laboratory and field.

MATERIALS AND METHODS

Chemicals and biocontrol agents tested. Seventeen chemicals and five biocontrol agents were tested for their efficacy for control of *Xanthomonas cucurbitae* (Table 3.1). Nine of the 17 tested chemicals were copper compounds.

Laboratory test of chemicals. Eleven chemicals (Table 3.2) with the known bactericidal effects were tested in the laboratory to determine effective concentration for 50% reduction in *X. cucurbitae* cell multiplication (EC_{50}) in nutrient broth (NB) and casitone yeast extract broth (CYE). NB was prepared by adding 8 g of Difco ^{TM} nutrient broth to 1,000 ml distilled water. CYE contained 1.7 g casitone, 2 g glucose, and 0.35 g yeast extract in 1,000 ml of distilled water. The spectrophotometric method was used to measure the cell density as described by Dominguez, et al (2001).

Ten isolates of *X. cucurbitae* from different parts of Illinois (Calhoun, Champaign, DeKalb, Henry, Jefferson, Kankakee, McLean, Moultrie, Ogle, and Putnam Counties) were used in this study. Each isolate was cultured separately on Luria Bertani agar (LB) medium in Petri plates, incubated in the dark at 28 ± 1^{o}C for 3 days. Colonies were washed with sterile distilled water (SDW), and the bacterial suspension was adjusted to 10^8 CFU/ml using a spectrophotometer (Smart Spec 3000, Bio-Rad, Philadelphia, PA).

The efficacy tests of the chemicals were in NB and CYE media in sterile 24-well tissue culture plates (BD Labware, Franklin Lakes, NJ). Media were freshly prepared each time and amended with the chemical, before being pipetted into the 24-well tissue culture plates. First the lethal dose (EC_{100}) of each chemical was determined in nutrient agar in Petri dishes, the concentration that controls the growth of every single colony. For each chemical, five different concentrations were tested. The minimum concentration chosen reduced less than 50% cell multiplication from control and maximum concentration was the EC_{100} ppm. Fifty microliters of the bacterial suspension was added to 2 ml of the broth in each well. The plates were placed on a shaker at

100 rpm, at 30^{o}C for 24 hr as described by Mahmood et al. (2011). Each test included two controls, one with non-chemical amended culture medium with bacterial suspension, and the other with chemical amended but without bacterial suspension. Bacterial cell density was assessed using the spectrophotometer at OD600 nm (optical density), as described by Dominguez et al. (2000). Each isolate was tested twice with four replications.

The accuracy of measuring bacterial cell density by the spectrophotometer was verified by using agar-count method. The EC_{50} value of Kocide was tested with both spectrophotometric and agar-count methods. All previously-mentioned 10 isolates of *X. cucurbitae* were tested. The bacterial suspensions were prepared in the same way as in spectrophotometric method. Then, the bacterial suspension of 10^8 CFU/ml was diluted to 10^3 CFU/ml. Nutrient agar (NA) medium was amended with Kocide. Using a sterilized bent glass rod, 100 µl of 10^3 CFU/ml was spread onto NA medium in Petri dishes and dishes were incubated in the dark at 28 ± 1^{o}C. Colony forming units were calculated 3 days after incubation.

Laboratory test of biocontrol agents. The same 10 isolates of *X. cucurbitae* used for testing chemicals were also used for testing biocontrol agents. Each isolate was grown separately on LB in Petri dishes in the dark at 28 ± 1^{o}C for 3 days. Colonies were washed with SDW and the suspension was adjusted to 10^8 CFU/ml using the spectrophotometer. Using a sterilized bent glass rod, 50 µl of the bacterial suspension was spread onto LB in Petri dishes.

Biocontrol agents were grown in CYE (100 µl of biocontrol agent in 30 ml broth) in a 200-ml flask on a shaker with 100 rpm at 30^{o}C for 72 hr (Fernando et al., 2005). Using the methods

described by Fernando et al. (2005), Loper et al. (1991), and Russo et al. (2008), a 1-cm diameter filter-paper was soaked in CYE with the biocontrol agent for 5 sec, briefly dried on a sterilized paper towel in a sterile hood, and placed onto the surface of LB with *X. cucurbitae*. For Regalia, a 1-cm filter-paper was soaked in the manufactured product directly and then placed on LB with *X. cucurbitae*. A filter-paper soaked in SDW was used as control. Clear zones of inhibition were measured after 3 days. Each isolate was tested twice with four replications (four plates).

Field trials. Field trials were conducted at the University of Illinois Vegetable and Research Farm in Champaign, IL in 2012 and 2013 to evaluate effectiveness of the chemicals and biocontrol agents for control of bacterial spot in pumpkin. The studies included a trial of chemicals in 2012, a trial of biocontrol agents in 2012, and a combined trial of chemicals and biocontrol agents in 2013. The soil was a silt clay loam with pH 6.5. During the season, weeds were controlled by cultivation and hand-weeding. Cucumber beetles (*Acalymma vittatum* and *Diabrotica undecimpunctata*) and other insects were managed by applying permethrin (Perm-Up 3.2 EC, 4 fl oz/A) and bifenthrin (Capture 2 EC, 5 fl oz/A).

Chemical trial in 2012. Seeds of jack-o-lantern pumpkin 'Howden' were sown on 6 June. Seeds were sown 46 cm apart in single-row plots, 6 m long. The design was randomized complete block design with three replications, and the plots were spaced 10 m apart. Plants were inoculated twice with *X. cucurbitae* (5×10^7 CFU/ml) mixed with carborundum powder (0.5 g carborundum/L inoculum) on 23 July (beginning of flowering stage) and 13 August (fruit setting stage) using a backpack sprayer. The first inoculation was for establishing infection in leaves and the second inoculation mainly aimed for establishing fruit infection.

Chemicals were spray-applied with a backpack sprayer using 50 gallons of water per acre. The first application of the chemicals was either on 19 July [4 days prior to the first inoculation (pre-inoculation spray)] or on 26 July [3 day after inoculation (post-inoculation spray)]. Then, the chemicals were applied at 7-day intervals until 6 September. Bacterial spots were observed on leaves on 2 August (10 days after the first inoculation) and severity of infection increased as the season progressed. Bacterial spots were observed on fruit on 17 August (25 days after the first inoculation), when fruit were approximately 15 cm in diameter. Incidence (percent symptomatic leaves) and severity (percent symptomatic area of leaves) of bacterial spot were assessed on 25 August and 11 September. Incidence and severity of bacterial spot on fruit were assessed on 23 September. Severity of the disease on leaf and fruit was evaluated using a scale of 0-7, 0 = 0; 1 = 1; 2 = 2-5; 3 = 6-15; 4 = 16-30; 5 = 31-50; 6 = 51-70; and 7 = 71-100% of leaf or fruit surface with lesions. In each plot, five vines were randomly selected and leaves of these vines and all of the fruit in the plot were evaluated for the occurrence of bacterial spot.

***Biocontrol agent trial 2012*.** Jack-o-lantern pumpkin 'Howden' seedlings were grown in a greenhouse and 10-day-old seedlings were transplanted on 25 June. Ten seedlings were transplanted in single-row plots, 4.5 m long. The design was randomized complete block design with three replications, and the plots were spaced 10 m apart. Plants were inoculated twice with *X. cucurbitae* (5×10^7 CFU/ml) on 30 July (beginning of flowering stage) and 13 August (fruit setting stage), as previously described. Biocontrol agents were applied with a backpack sprayer using 50 gallons of water per acre. First application of biocontrol agents was on 26 July [4 days prior to inoculation (pre-inoculation spray)] and continued at 7-day intervals until 6 September. Bacterial spots were observed on leaves on 19 August (20 days after the first inoculation) and

severity of infection increased as the season progressed. Bacterial spots were observed on fruit on 29 August (30 days after the first inoculation), when fruit were approximately 15 cm in diameter. The scale used to assess severity of bacterial spot on leaves and fruit was the same as previously described. In each plot, five vines were randomly selected and leaves of the vines and all of the fruit in the plot were evaluated for occurrence of bacterial spot.

Chemical and biocontrol agent trial in 2013. Seeds of jack-o-lantern pumpkin 'Howden' were sown on 4 June. The trial was performed in a randomized complete block design as described in the chemical trial in 2012. Plants were inoculated three times with *X. cucurbitae* (5×10^7 CFU/ml) on 27 July (beginning of flowering stage), 19 August (fruit setting stage), and 2 September (color change of fruit from green to orange). Chemicals were spray-applied either on 24 July (pre-inoculation spray) or on 30 July (post-inoculation spray). All biocontrol agents were applied on both 24 July and 30 July. From 6 August until 10 September, all chemicals and biocontrol agents were applied at 7-day interval. Bacterial spots were observed on leaves on 4 August (8 days after the first inoculation) and severity of infection increased as the season progressed. Bacterial spots were observed on fruit on 25 August (29 days after the first inoculation). Incidence and severity of bacterial spot on leaves was assessed on 15 September and on fruit on 25 September. In each plot, leaves of five randomly selected vines, and all fruit, were evaluated for the occurrence of bacterial spot. Disease severity on fruit was assessed by evaluating five randomly-selected fruit in each plot.

Data analysis. All statistical analysis was performed using SAS 9.3 (SAS Institute Inc. Cary, NC). EC_{50} values of chemicals were analyzed using macro in SAS 9.3 according to the method

described by Wise et al., (2008). Also, comparison of EC_{50} values for all treatments in two media were made using PROC GLM. Correlation of EC_{50} and EC_{100} values between NB and CYE media were determined in Ms-Excel. Comparison of cell density determined by the spectrophotometer method and the agar-count method was carried out using PROC GLM. For the field data, homogeneity of variances was tested using the Brown-Forsyth test and were checked for normality to meet valid assumptions. Data were analyzed using ANOVA in PROC MIXED and macro pdmix800 to indicate mean letter separation (Saxton, 1998). Field data of 2012 and 2013 were analyzed separately because in 2012 we had separate trials for chemicals and biocontrols, whereas in 2013 all chemicals and biocontrols were tested in the same trial.

RESULTS

Laboratory test of chemicals. Cell density of *X. cucurbitae* measured by the spectrophotometric method and number of CFU determined by the agar-count method were not significantly different (P = 0.3609) from each other. There were significant difference between the EC_{50} values of all 11 chemicals in NB and CYE medium (Table 3.2). EC_{50} value of fungicide mancozeb (Dithane) was the lowest in both media, followed by those of streptomycin (AgriMycin) and oxytetracycline (Mycoshield). EC_{50} value of cuprous oxide (Nordox) was the greatest in both media (Table 3.2). Overall EC_{50} values of the chemicals in NB and CYE were highly correlated (R^2 = 0.97). The EC_{100} values of the chemicals tested ranged from 1 ppm mancozeb (Dithane) to 175 ppm cuprous oxide (Nordox) in NB, and from 1 ppm mancozeb (Dithane) to 125 ppm cuprous oxide (Nordox) in CYE (Table 3.2). EC_{100} values of the chemicals in NB and CYE were also highly correlated (R^2 = 0.97).

There were significant differences (P = 0.0003) in the EC_{50} values of chemicals measured in NB medium (Table 3.2). All EC_{50} values were significantly different from each other, except EC_{50} values of Cufrofix vs Kasumin, SciEX83-3S vs SciEX83-4S, and AgriMycin vs Dithane vs Mycoshild that were not significantly different from each other. Similarly, EC_{50} values of the chemicals measured in CYE were significantly different (P = 0.0003). However, there were not significant differences in the EC_{50} values of Cuprofix vs Kocide, Cuprofix vs Kasumin, SciEX83-3S vs SciEX83-4S, and AgriMycin vs Dithane vs Mycoshield.

Laboratory test of biocontrol agents. All five tested biocontrol agents prevented multiplication of *X. cucurbitae* cells around the filter disc.

Field trials. Results of the field trials were as follows.

***Chemical field trial in 2012*.** Severity of bacterial spot on leaves was significantly (P = 0.0001) lower in the plots sprayed with chemicals compared to that of control plots (Table 3.3). On 25 August, severity of bacterial spot on leaves in treated plots ranged from 1.50 to 2.56% whereas severity of the disease on leaves in control plots was 8.07%. On 11 September, severity of bacterial spot on leaves in treated plots ranged from 2.80 to 6.93% compared to control plots with severity of the disease of 11.48 % (Table 3.3). The lowest severity of bacterial spot on leaves was in the plots sprayed with Mycoshield.

Incidence and severity of bacterial spot on fruit in treated plots were lower than those of control plots (Table 3.3). Incidence of the disease in fruit in treated plots ranged from 11.1 to 82.2% compared to that of control plots 88.9%. Severity of the disease on fruit in treated plots ranged

from 0.07 to 3.97%, whereas severity of the disease was 4.33% in control plots. The lowest incidence of bacterial spot on fruit (11.1%) was in the plots that received pre-inoculation spray of Mycoshield. The lowest severity of bacterial spot on fruit (0.07%) was in the plots received pre-inoculation spray of Phyton (Table 3.3).

Biocontrol agent field trial in 2012. Severity of bacterial spot on leaves was significantly (P = 0.0001) lower in the plots sprayed with biocontrol agents than control plots (Table 3.4). Incidence of bacterial spot on fruit in treated plots ranged from 0.0% (Regalia) to 59.4% (Actinovate), compared to the incidence of the disease in control plots (88.9%). Severity of the disease on fruit in treated plots ranged from 0.0% (Regalia) to 3.97% (CX-9030 + Cueva), whereas severity of the diseases in control plots was 4.33% (Table 3.4).

Chemical and biocontrol agents field trial in 2013. There were significantly lower severity of bacterial spot on leaves (P = 0.0003) and lower incidence of bacterial spot on fruit (P = 0.0003) compared those of control plots (Table 3.5). Also, severity of bacterial spot on fruit in most of the treated plots was significantly lower than that of control plots. There were no statistically significant differences in incidence and severity of bacterial spot on leaves and fruit in the plots that received pre-inoculation treatments and post-inoculation treatments. Lowest disease severity on leaves, disease incidence on fruit, and disease severity on fruit were in the plots that received pre-inoculation treatment of Cuprofix, pre-inoculation treatment of Badge, and pre-inoculation treatment of ActiGard plus Kocide, respectively (Table 3.5).

DISCUSSION

There were significant differences between the EC_{50} values of the chemicals tested in NB and CYE. But, the values from the two culture media were highly correlated. Thus, using either culture medium to assess effective concentration of the chemical would be reliable. Among all tested chemicals with copper active ingredients, the EC_{50} values measured was significantly lower in CYE than NB, but was opposite for Dithane, a non-copper compound. Similar results have been reported by Menkissoglu and Lindow (1991). They studied effectiveness of copper in culture media and reported that type of media play an important role in the availability of the free cupric ions in the medium. Copper resistant to several *Xanthomonas* spp. has been reported. So, in addition to the preliminary information for the use of chemical in the field, laboratory test is also useful for the copper resistant analysis. There were no significant differences in EC_{50} values among the effective chemicals Dithane, Agrimycin, and Mychoshield in both media suggest even Dithane have strong bactericidal effects like bactericides Agrimycin and Mychoshield.

Among chemicals tested, oxytetracycline (Mycoshield), copper sulfate (Cuprofix), and copper hydroxide (Kocide) were effective in suppressing *X. cucurbitae* in both laboratory and field. Mancozeb (Dithane) was more effective for control of *X. cucurbitae* in the laboratory than in the field. *Bacillus subtilis* (Serenade) was effective in suppressing *X. cucurbitae* in both laboratory and field, whereas Regalia was more effective in the field than in the laboratory. Overall, testing chemicals and biocontrols in the laboratory provides some preliminary information for their effectiveness in the field. But without field evaluation of efficacy of chemicals for control of *X. cucurbitae*, laboratory tests alone would not be reliable.

Although the incidence and severity of bacterial spot on leaves and fruit were significantly lower in the plots treated with chemicals and biocontrol agents, none of the chemicals or biocontrol agent tested in this study was highly effective in controlling the disease in pumpkin. Similar results have been reported by Lange and Smart (2013), indicating that Cueva, Regalia, Kocide, and ActiGard reduced incidence of black rot (*X. campestris* pv. *campestris*) in cabbage but did not control it effectively.

Severity of bacterial spot in leaves was not much in both 2012 and 2013, but the incidence of fruit infection was high. *X. cucurbitae* in leaf may be control by the forced spray-application of the treatments over the foliage, but once the fruit get infected by *X. cucurbitae* even the forced spray-application could not penetrate the thick pumpkin skin. Also the smooth skin of the pumpkin may retain the chemical for less time than foliage. Using adjuvants with chemicals may increase efficacy of chemical for control of fruit infection by *X. cucurbitae*. Additional investigation on chemical treatment of pumpkin plant for control of *X. cucurbitae* is needed.

In this study, application of chemicals began at flowering stage of plant growth. By that time, infection of plants by natural inoculum of *X. cucurbitae* may had taken place, although no symptoms were visible yet. It is possible that these treatments could have worked as better control of the bacterial spot if application of chemicals had begun from earlier stage of plant growth. Further studies are needed to determine disease development of bacterial spot from natural inoculum in the fields.

Integrated approaches of chemicals and/or biocontrol agents can be used for the effective management of the *X. cucurbitae* in pumpkin. In our treatment list we have a chemical ActiGard and biocontrol agent Regalia, both triggers the plant defense system. Application of these compounds from the early season and together with sprays of copper compound like Kocide and Cuprofix should be helpful to control the disease. In addition, a 4- or 5-year crop rotation with non-cucurbit plants and application of the most effective chemicals (e.g., Kocide, mycoshield) may further reduce yield losses by bacterial spot. For developing effective strategies for management of bacterial spot, identifying/developing resistant cultivars of pumpkin, determining effective crop rotation, and timely application of chemicals should be investigated.

TABLES AND FIGURES

Table 3.1. Chemicals and biocontrol agents tested for control of *Xanthomonas cucurbitae* in the laboratory and fields during 2012-2013.

Compounds	Active ingredients	FRAC code[w]
Chemical		
ActiGard 50 WG	acibenzolar-s-methyl	P[x]
Agion E	copper	M1[y]
Badge X2 DF	copper oxychloride + copper hydroxide	M1
Cuprofix Ultra 40 DF	copper sulfate	M1
Kocide-3000 46.1 DF	copper hydroxide	M1
Nordox 75 WG	cuprous oxide	M1
Phyton-016B	copper sulfate pentahydrate	M1
SciEX83-3S	copper, silver, and zinc salts	M1
SciEX83-4S	copper, silver and zinc salts	M1
AgriMycin 17 WP	streptomycin	25
Kasumin 2L	kasugamycin	24
Mycoshield 40 WSP	oxytetracycline	41
Cueva FL	copper octanoate	M1
Dithane 75 DF	mancozeb	M3[y]
Tanos 50 DWG	famoxadone + cymoxanil	27
Quintec 2.08 SC	quinolin/quinexyfen	13
Milstop SP	potassium biocarbonate	NC[z]
Biocontrol agents		
Actinovate AG	*Streptomyces lydicus*, strain WYEC 108	NC

Cx-9030 WDG	*Bacillus amyloliquefacien*, strain D747	44
Serenade ASO	*Bacillus subtili*, strain QST 713	NC
Sonata ASO	*Bacillus pumilus*, strain QST 2808	NC
Regalia	extract from *Reynoutria sachalinensis*	P

[w] FRAC = fungicide resistance action committee (http://ipm.ifas.ufl.edu/resources/success_stories/T&PGuide/pdfs/Appendices/Appendix6-FRAC.pdf)

[x] P = host plant defense induction.

[y] (M1 and M3) = multi-site contact activity.

[z] NC = not classified.

Table 3.2. Effective concentration of chemicals for 50% and 100% reduction in multiplication of *Xanthomonas cucurbitae* cells (EC_{50} and EC_{100}) in culture media.

	EC_{50} (ppm)				EC_{100} (ppm)	
Chemical	**NB[w]**	**CYE[x]**	**LSD**	**Pr > F**	**NB**	**CYE**
streptomycin (AgriMycin 17 WP)	0.40 (G)[y] a[z]	0.31 (G) b	0.07	0.0066	2.0	1.0
copper oxychloride + copper hydroxide (Badge X2 DF)	22.82 (C) a	15.19 (C) b	1.77	0.0001	55	25
copper sulfate (Cuprofix Ultra 40 DF)	11.33 (E) a	9.83 (DE) b	0.94	0.0034	60	40
mancozeb (Dithane 75 DF)	0.17 (G) b	0.23 (G) a	0.04	0.0033	1.0	1.0
kasumagycin (Kasumin 2L)	13.16 (E) a	9.29 (E) b	1.41	0.0001	50	35
copper oxychloride (Kocide-3000 46.1 DF)	16.23 (D) a	11.33 (D) b	1.72	0.0001	55	35
oxytetracycline (Mycoshield 40 WSP)	0.40 (G) a	0.24 (G) b	0.07	0.0001	2.0	1.5
cuprous oxide (Nordox 75 WG)	64.53 (A) a	38.87 (A) b	6.04	0.0001	175	125
copper sulpate pentahydrate (Phyton-016B)	33.07 (B) a	21.76 (B) b	3.84	0.0001	55	65
copper, silver, and zinc (SciEX83-3S)	7.94 (F) a	5.70 (F) b	1.44	0.0033	20	15
copper, silver, and zinc (SciEX83-4S)	7.80 (F) a	5.48 (F) b	1.03	0.0001	20	10

[w] Nutrient broth

[x] Casitone yeast extract broth

[y] In each column values with upper case letter in common are not significantly different from each other according to Fisher's protected LSD ($P = 0.05$).

[z] In each row values with a lower case letter in common are not significantly different from each other according to Fisher's protected LSD ($P = 0.05$).

Table 3.3. Incidence and severity of bacterial spot caused by *Xanthomonas cucurbitae* in pumpkin following application of chemicals in the field in 2012.

Treatment, product rate/A[t]	Application of chemical	Severity of bacterial spot on leaves (%)[u]		Bacterial spot on fruit (23 September)	
		25 August	11 September	Incidence (%)[v]	Severity (%)[w]
Control Check	---------	8.07 a[x]	11.48 a	88.9 a	4.33 a
ActiGard 50 WG, 1 oz + Kocide-3000 46.1 DF, 1.25 lb	Pre[y]	1.74 d-f	3.13 lm	33.3 c-g	0.20 gh
	Post[z]	1.72 d-f	3.17 lm	82.2 ab	0.73 f-h
Agion E, 3 gallon	Pre	2.01 cd	3.57 j-l	58.3 a-f	0.83 e-h
	Post	2.02 d-f	4.05 g-j	72.2 a-d	1.23 c-h
AgriMycin 17 WP, 0.5 lb	Pre	2.06 cd	4.33 f-i	46.7 a-g	3.97 ab
	Post	2.01 d-f	6.93 b	60.0 a-f	1.13 c-h
Badge X2 DF, 1.25 lb	Pre	1.87 c-f	4.73 e-g	25.0 e-g	0.93 d-h
	Post	1.81 c-f	3.75 h-l	80.5 ab	3.60 a-e
Cuprofix Ultra 40 DF, 2 lb	Pre	1.82 c-f	3.72 i-l	58.3 a-f	2.31 a-h
	Post	1.76 d-f	4.93 ef	53.3 a-g	1.80 a-h
Dithane 75 DF, 2.5 lb + Kocide-3000 46.1 DF, 1.25 lb	Pre	1.97 c-e	3.90 h-k	72.2 a-d	3.77 a-c
	Post	1.75 d-f	4.08 g-j	75.5 a-c	2.91 a-g

Table 3.3. Continued.

Treatment, product rate/A[t]	Application of chemical	Severity of bacterial spot on leaves (%)[u]		Bacterial spot on fruit (23 September)	
		25 August	11 September	Incidence (%)[v]	Severity (%)[w]
Kasumin 2L, 1 qt	Pre	1.87 c-f	4.80 ef	60.0 a-e	0.13 h
	Post	2.00 d-f	5.88 cd	44.4 a-g	1.43 a-h
Kocide-3000 46.1 DF, 1.25 lb	Pre	2.01 d-f	5.33 de	69.0 a-e	1.20 b-h
	Post	2.18 bc	6.44 bc	64.4 a-f	3.37 a-f
Mycoshield 40 WSP, 1 lb	Pre	1.50 f	3.23 k-m	11.1 g	0.20 gh
	Post	1.60 ef	2.80 m	61.1 a-f	1.43 a-h
Nordox 75 WG, 1.25 lb	Pre	1.98 c-e	4.09 g-i	55.6 a-g	1.20 b-h
	Post	2.08 cd	5.00 ef	64.4 a-f	3.37 a-f
Phyton-016B, 25 fl oz	Pre	2.18 bc	5.20 de	23.3 fg	0.07 h
	Post	2.00 d-f	6.07 c	62.2 a-f	1.97 a-h
Tanos 50 DWG, 10 oz + Kocide-3000 46.1 DF, 1.25 lb	Pre	1.80 c-f	4.07 g-j	26.7 d-g	2.27 a-h
	Post	2.56 b	4.43 f-h	42.2 b-g	2.63 a-h

Table 3.3. Continued.

Treatment, product rate/A[t]	Application of chemical	Severity of bacterial spot on leaves (%)[u]		Bacterial spot on fruit (23 September)	
		25 August	11 September	Incidence (%)[v]	Severity (%)[w]
Quintec 2.08 SC, 6 fl oz + Kocide-3000 46.1 DF, 1.25 lb	Pre	2.07 cd	6.47 bc	69.4 a-e	3.03 a-f
	Post	1.97 c-e	5.23 de	42.2 b-g	3.73 a-d
LSD (P = 0.05)		0.53	0.70	45.0	2.78

[t] Product rate per acre.

[u] Percent symptomatic area of leaves.

[v] Incidence = percent symptomatic fruit.

[w] Severity = percent affected area of fruit.

[x] In each column values with a letter in common are not significantly different from each other according to the Fisher's protected LSD (P = 0.05).

[y] Pre = plants were treated on 19 July, 26 July, 2 August, 9 August, 16 August, 23 August, 30 August, and 6 September.

[z] Post = plants were treated on 26 July, 2 August, 9 August, 16 August, 23 August, 30 August, and 6 September.

Table 3.4. Incidence and severity of bacterial spot caused by *Xanthomonas cucurbitae* in pumpkin following application of biocontrol agents in the field in 2012.

Treatment, product rate/A[v]	Severity of bacterial spot on leaves (%)[w]		Bacterial spot on fruit (23 September)	
	11 September	23 September	Incidence (%)[x]	Severity (%)[y]
Control check	11.49 a[z]	19.29 a	88.9 a	**4.33 a**
Actinovate AG, 12 oz	5.10 b	6.40 b	59.4 ab	**2.63 ab**
Cx-9030 WDG, 3 lb + Cueva FL, 2 qt	3.50 cd	4.83 c	54.7 a-c	**3.97 a**
Milstop SP, 5 lb + Serenade ASO, 6 qt	4.23 bc	5.40 bc	27.8 bc	**2.20 ab**
Regalia, 1 qt	3.23 cd	4.43 c	0.0 c	**0.00 b**
Serenade ASO, 6 qt	2.53 d	4.00 c	16.7 bc	**0.23 b**
Sonata ASO, 4 qt	3.17 cd	4.37 c	44.4 a-c	**0.27 b**
LSD (P = 0.05)	1.34	1.45	55.1	**3.46**

[v] Product rate per acre. Compounds were spray-applied on a weekly schedule from 19 July until 6 September.

[w] Percent symptomatic area of leaves.

[x] Incidence = percent symptomatic fruit.

[y] Severity = percent symptomatic area of fruit.

[z] In each column values with a letter in common are not significantly different from each other according to the Fisher's protected LSD (P = 0.05).

Table 3.5. Incidence and severity of bacterial spot caused by *Xanthomonas cucurbitae* in pumpkin following application of chemical and biocontrol agents in the field in 2013.

Treatment, product rate/A[s]	Application of chemical	Severity of bacterial spot on leaves (%)[t]	Bacterial spot on fruit (25 September)	
		15 September	Incidence (%)[u]	Severity (%)[v]
Control Check	-------	2.01 a[w]	71.7 a	3.00 a
ActiGard 50 WG, 1 oz + Kocide-3000 46.1 DF, 1.25 lb	Pre[x]	0.44 gh	31.5 d-g	0.41 g
	Post[y]	0.82 c-h	41.7 b-f	0.77 e-g
AgriMycin 17 WP, 0.5 lb	Pre	0.89 c-h	35.1 d-g	1.87 a-e
	Post	0.92 c-h	46.3 b-e	1.45 b-g
Badge X2 DF, 1.25 lb	Pre	0.76 c-h	25.4 g	1.75 b-f
	Post	0.86 c-h	43.3 b-f	0.83 d-g
Cuprofix Ultra 40 DF, 2 lb	Pre	0.37 h	34.8 d-g	0.66 fg
	Post	0.69 d-h	43.1 b-f	1.04 b-g
Dithane 75 DF, 2.5 lb + Kocide-3000 46.1 DF, 1.25 lb	Pre	0.69 d-h	51.2 bc	1.33 b-g
	Post	1.05 b-e	44.6 b-f	1.66 b-f
Kasumin 2L, 1 qt	Pre	0.48 f-h	44.4 b-f	1.46 b-g
	Post	0.46 f-h	44.4 b-f	1.33 b-g

Table 3.5. Continued.

Treatment, product rate/A[s]	Application of chemical	Severity of bacterial spot on leaves (%)[t]	Bacterial spot on fruit (25 September)	
		15 September	Incidence (%)[u]	Severity (%)[v]
Kocide-3000 46.1 DF, 1.25 lb	Pre	0.98 c-g	42.2 d-f	0.66 fg
	Post	0.78 c-h	42.1 b-f	0.77 e-g
Mycoshield 40 WSP, 1 lb	Pre	0.68 d-h	30.7 e-g	1.04 b-g
	Post	0.86 c-h	44.1 b-f	1.90 a-d
Nordox 75 WG, 1.25 lb	Pre	0.87 c-h	32.1 d-g	1.00 e-g
	Post	1.11 b-d	42.9 b-f	1.30 b-g
Phyton-016B, 25 fl oz	Pre	0.89 c-h	36 c-g	0.66 fg
	Post	0.91 c-h	47.4 b-d	2.09 a-c
Tanos 50 DWG, 10 oz + Kocide-3000 46.1 DF, 1.25 lb	Pre	0.55 e-h	30.0 fg	1.20 b-g
	Post	0.90 c-h	40.0 c-g	1.63 b-f
Quintec 2.08 SC, 6 fl oz + Kocide-3000 46.1 DF, 1.25 lb	Pre	0.77 c-h	32.8 d-g	1.46 b-g
	Post	1.12 b-d	40.4 b-g	1.30 b-g

Table 3.5. Continued.

Treatment, product rate/A[s]	Application of chemical	Severity of bacterial spot on leaves (%)[t]	Bacterial spot on fruit (25 September)	
		15 September	Incidence (%)[u]	Severity (%)[v]
SciEx83-3S (1:232)	Pre	0.70 c-h	45 b-f	1.20 b-g
	Post	0.90 c-h	35.7 d-g	1.54 b-g
Actinovate AG, 12 oz	Pre	0.58 d-h	40.0 c-g	0.83 d-g
Cx-9030 WDG, 3 lb + Cueva FL, 2 q[z]	Pre	0.54 e-h	42.5 b-f	1.00 c-g
Milstop SP, 5 lb + Serenade ASO, 6 qt	Pre	1.00 b-f	40.0 c-g	1.63 b-f
Regalia, 1 qt	Pre	0.46 f-h	35.8 c-g	2.20 ab
Serenade ASO, 6 qt	Pre	0.73 c-h	43.5 b-f	1.50 b-g
Sonata ASO, 4 qt	Pre	0.93 c-h	44.7 b-f	1.05 b-g
LSD (P = 0.05)		0.55	16.1	1.18

[s] Product rate per acre.
[t] Percent symptomatic area of leaves.
[u] Incidence = percent symptomatic fruit.
[v] Severity = percent affected area of fruit.
[w] In each column values with a letter in common are not significantly different from each other according to the Fisher's protected LSD (P = 0.05).
[x] Pre = plants were treated on 24 July, 30 July, 6 August, 13 August, 20 August, 27 August, 3 September, and 10 September.
[y] Post = plants were treated on 30 July, 6 August, 13 August, 20 August, 27 August, 3 September, and 10 September.
[z] Cueva was sprayed at the rate of 1 qt/acre for first three applications (24 July, 30 July, 6 August) and 2 qt/acre for the remaining applications (13 August, 20 August, 27 August, 3 September, 10 September).

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