THE EPIDEMIOLOGY OF *Xanthomonas campestris* pv. *vitians,* CAUSAL ORGANISM OF BACTERIAL LEAF SPOT OF LETTUCE

By

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By

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Several factors were investigated that may be involved in the epidemiology of Bacterial Leaf Spot (BLS) caused by *Xanthomonas campestris* pv. *vitians* in lettuce. These include the effect of temperature on development of disease, the role of epiphytic populations on different plant species, the effect of wounding on disease development, and the determination of internal dynamics in selected plant species. Dr. Ken Pernezny contributed data on the infectivity of *X. c.* pv. *vitians*. Temperature studies were performed on cos lettuce plants. In this study, optimal disease development occurred at 22.7ºC. Wounding experiments were performed on lettuce, the known host plant, as well as nine other plant species from seven different plant families. Non-wounded and wounded plants were statistically different from each other in which wounding was associated with less disease compared to non-wounded plants. A second study involved infiltrating lettuce, tomato, pepper, cilantro, parsley, and beets with 10^5 cells per gram of leaf tissue and assaying for internal populations of *X. c.* pv. *vitians.* Populations of *X. c.*

pv. *vitians* followed a typical bacterial growth curve in lettuce, pepper, and cilantro. In trial 1, populations peaked at $3.6x10^8$, $5.8x10^6$ and $1.6 x10^5$ CFU/cm^2 in those crops, respectively, then dropped to $1.0x10^6$, $3.2 x10^5$ and $3.3 x10^3$ CFU/cm^2 of leaf tissue on the final day of sampling. Bacterial populations in tomato increased slightly to $2.1x10^5$ CFU/cm^2 and remained there. Bacterial populations in beet and parsley followed a different growth curve by making a drastic dip at Day 0 of the sampling period and growing slowly to $2.0 x10^5$ and $8.4 x10^6$ CFU/cm^2. Respectively, in Trial 2, bacterial populations in lettuce and pepper again followed a curve with peaks at $1.35x10^8$ and 5.5 x 10^6 CFU/cm^2, respectively. Bacterial populations in tomato rose to $3.8 x 10^5$CFU/cm^2. Bacterial populations in parsley, cilantro, and beet had high populations of $1.85 x 10^5$, $1.3 x 10^5$, and $5.4 x 10^3$ CFU/cm^2 , respectively. Epiphytic populations were measured in a field trial on the Pine Acres Research Center in Marion County, FL. Cos lettuce, tomato, parsley, cilantro, endive and beets were evaluated for epiphytic populations of *X. c.* pv. *vitians*. Lettuce was shown to support higher epiphytic populations for compared to all other host species 5 out of 6 weeks that were sampled. Of these crops tomato was the only one to support populations of *X. c.* pv. *vitians* in the final sampling date. Statistically higher populations were found on lettuce, tomato, and endive compared to cilantro, parsley and beets at two Weeks After Inoculation (WAI). This research shows that pepper and tomato may harbor relatively higher populations of *X. c.* pv. *vitians* making them possible alternative hosts. Optimum temperature for development of BLS on lettuce was determined to be 22.7ºC. Wounding data were inconclusive.

CHAPTER 1
LITERATURE REVIEW

The Host

Lettuce, (*Lactuca sativa* L.) including cos or romaine lettuce, belongs to the *Asteraceae* (*Compositae*) or daisy family. This is the largest dicotyledonous family in the plant kingdom. Notable leafy crop species within the *Asteraceae* family include lettuce, chicory, and endive, as well as, other lesser known plants such as fuki and oyster plant (Ryder 1999). Lettuce is grown commercially in many parts of the world, with important industries in North America, Western Europe, the Mediterranean, Australia, and parts of Asia. In 2000, the average American consumed 33 pounds of lettuce (Glaser 2001; Rupp 1987). It is a staple in home gardens in many parts of the world and is a good source of dietary fiber, vitamins, and minerals.

Production

Production of lettuce is limited to temperate and subtropical parts of the world where temperatures are not too warm. Daily temperature averages conducive for growth of lettuce are in the 10-20ºC's (50-60ºF) range, because heading and stalk production is enhanced when temperatures are 21-27ºC (70-80ºF) (Ryder 1999; Stephens 1988; Yamaguchi 1983). Summer and fall lettuce crops in California take 60-80 days to reach maturity, while those grown in winter and spring take 90-145 days (Yamaguchi 1983). The world's largest commercial production takes place in China, followed by the USA (Glaser 2001). Most production in the United States takes place in Arizona and

California, with New Mexico, New Jersey, Colorado, and Florida being relatively minor production areas (Rupp 1987).

Crisphead lettuce is the most economically important leaf crop with total production in the United States being 3,289,070 metric tons in 2001 (Agriculture 2001). Production of leaf lettuce and cos lettuce were 532,240 and 739,030 metric tons, respectively (Agriculture 2001). Ninety-six percent of the production of commercial crisphead lettuce in the US occurs in California and Arizona (Glaser 2001). These two states also produce 98% of the United States' leaf and romaine lettuce (Glaser 2001).

Nutritional Value

Lettuce contributes to the diet by adding vitamins, minerals, fiber, and water (Tindall 1983). The main nutrients contributed by lettuce are vitamin A, calcium, and ascorbic acid. Cos and leaf types are the more nutritious of the lettuce types. In cos, there are 18 mg. of calcium, 4 mg ascorbic acid, and 1900 I.U. of vitamin A per 100 g edible tissue.

Taxonomy and Botany

The lettuce root system consists of a tap root with lateral roots forming along the entire length. Feeder roots are closer to the surface; therefore, nutrient and water absorption occur mainly near the soil surface. The leaves of lettuce are spirally arranged on a shortened stem, producing a rosette. Rosette formation is different for the different types of lettuce. Crisphead lettuce forms a rounded head, and butterhead types produce a more elongated head. With the onset of maturity, stem elongation occurs and reproductive development begins. The flowers are five-petalled, yellow and grow in dense clusters (Ryder 1999; Tindall 1983). In lettuce, the flowering event is referred to as "bolting" and it is induced by increases in nighttime temperatures.

Description of Horticultural Types of Lettuce

There are seven different types of lettuce: crisphead, butterhead, cos, leaf, Latin, stem, and oil-seed. Classification into type is dependent on leaf shape and size, degree of rosette, and head formation. Color and stem type may also be used but are considered less important in the classification of lettuce types (Ryder 1999).

Crisphead lettuce is described as having large firm heads. It is designated "iceberg" by growers. This lettuce type is large and weighs about 1 kg with six or seven outer leaves. Leaf texture can be highly crisp to less crisp depending on the cultivar. Outer leaves are either bright green or dull green, and the interior color may be white to creamy yellow. A subtype of crisphead includes Batavia lettuce. These are similar to the crisphead in growth and texture, but the final shape is smaller and less dense. Headweights of Batavia types are about 500g.

Butterhead lettuce is widely grown in Europe where there are two subtypes, winter and summer, based on growing season. In the United States, there are also two subtypes, but these are based on physical appearance and size. U.S. Butterhead subtypes are the Boston and Bibb.

Cos, also known as romaine, has elongated leaves that range in color from yellow to dark green. Many of the leaves tend to be a dark green because of the open growth habit of the plant. This allows for the increased production of chlorophyll. Romaine heads weigh up to 750g (Stephens 1988).

Cultivars of leaf lettuce vary considerably in appearance. The leaves may be broad or lobed and many times, the leaf margins are frilled. Frilled varieties are mainly grown in the home garden. They range in color from yellow to dark green. The rosettes may be closed in a bunch or flat and open.

Latin lettuce is grown in Europe, the United States, and in South America. Cultivars of Latin lettuce have an upright growth habit with elongated leaves similar to the cos type. They have a leaf texture like that of the bibb-type butterhead

Stem lettuce is also known as stalk or asparagus lettuce. This type of lettuce is grown for consumption of the stem which thickens and elongates. The leaves are long and narrow. This type is grown mainly in the Middle East and China.

Oil-Seed cultivars are grown beyond the rosette stage and bolt readily. These are about 50% larger than other types of lettuce, with the seed being collected and pressed for oil. The use of lettuce oil is an ancient practice and may represent the first commercial use of *Lactuca* spp.

Classification of *Xanthomonas campestris* pv. *vitians*

Bacteria are prokayotes which are normally single celled with genetic information not bound by a nuclear membrane (Agrios 1997). The genus *Xanthomonas* consists of only plant pathogens which usually produce yellow, water-insoluble pigments. Early classification of the Xanthomonads was based on morphological characteristics, biochemical, and physiological tests (Swings and Civerolo 1993). Brown (1918) first reported on the causal agent of bacterial leaf spot of lettuce (BLS). Based on strains collected from farms in Beaufort county, South Carolina. She proposed that the organism be named *Bacterium vitians*. In 1951 Elliott reported that three xanthomonas species existed which are *X. vitians*, *X. lactucae*, and *X. lactucae-scariolae* (Elliott 1951). In 1954, Burkholder (1954) further characterized bacterial strains of the three described species of *Xanthomonas* pathogenic on *Lactuca*. He proposed that these strains were not distinct species of *Xanthomonas*, but synonyms for *X. vitians*. By 1974, over 100 *Xanthomonas* sp. had been identified (Dye and Lelliot 1974). In 1974, Dye and Lelliot

proposed that they be grouped into five taxa, with the majority placed in the species *campestris*. Later, a new nomenclature was created. In this system, a pathovar was designated to distinguish the organisms based on pathogenicity on a host (Dye and Lelliot 1974). All pathovars were classified within the species *X. campestris* (Swings and Civerolo 1993). In 1995, Vauterin, *et al.* performed DNA-DNA hybridization studies, combined with biochemical and physiological tests such as carbon utilization using the Biolog GN microplate system, to reclassify strains within *Xanthomonas*. They found heterogeneity among strains of *X. c.* pv. *vitians.* Vauterin *et al.* (1995) identified two groups of *X. campestris* pv. *vitians* which they designated as type A and type B. The type B strains had high homology with *X. campestris* pv. *pelargonii* and *X. campestris* pv. *hederae*. They proposed that type B strains be renamed *Xanthomonas hortorum* pv. *vitians*. Furthermore, it was proposed that the *X. campestris* pv. *vitians* type A strain be renamed *Xanthomonas axonopodis* pv. *vitians.* This was proposed because of high homology with Group 9 strains which, include 34 *X. campestris* pathovars and *X. axonopodis* (Vauterin *et al.* 1995). However, as of February 2002,speculation as to the integrity of the type A strain remained, and it has been removed from the Belgian Co-Ordinated Collections of Micro-organisms (personal communication, Claudia Vereecke 2003). Further references to this organism in this thesis will refer to the lettuce bacterial leaf spot pathogen of lettuce as *Xanthomonas campestris* pv. *vitians*.

Characterization of *Xanthomonas campestris* pv. *vitians*

X. campestris pv. *vitians* is a strictly aerobic, Gram-negative, rod-shaped bacterium ranging in size from 0.2-0.8µm by 0.6-2.0µm (Pernezny *et al.* 1995; Toussaint 1999). It is non-sporulating with a single polar flagellum. It is a chemo-organotroph that is never fermentative, oxidase negative, and catalase positive. Acid is not produced from most

carbohydrates. No acid is produced in litmus milk (Brown 1918; Buchanan and Gibbons 1974; Burkholder 1954; Dye and Lelliot 1974; Tindall 1983). Sodium hippurate benzoate, oxalate, and tartrate are not utilized as carbon sources. Nitrates are not reduced and hydrogen sulfide is produced from cysteine. Acetoin and indole are not produced. Asparagine is not utilized as a sole carbon and nitrogen source. *X. campestris* pv. *vitians* does not hydrolyze starch (Toussaint *et al.* 2001).

Distribution

Since 1918, BLS has been reported from Italy (Pennisi and Pane 1990), Canada (Toussaint 1999), Venezuela (Daboin and Tortolero 1991), Turkey (Sahin 2000), Japan (Tsuchiya et al. 1981), and South Africa (Wallis and Joubert 1972). In the United States it has been found in California (Schroth et al. 1964), Ohio (Sahin and Miller 1998), New York (Burkholder 1954), and Florida (Pernezny *et al.* 1995). In Florida, BLS caused serious damage to lettuce crops in the winter of 1992-1993 and is remains a concern to lettuce farmers in the Everglades (Pernezny *et al*. 2001).

Bacterial Leaf Spot Symptoms

There are two discrete symptoms associated with BLS. The first include watersoaked, brown lesions that later turn black about 1-2 mm. in diameter occur. These lesions become V-shaped, translucent, and collapse (Toussaint 1999; Sahin et al. 1997; Sahin and Miller 1998). Lesions may expand along the veins of the plant (Sahin et al. 1997; Sahin and Miller 1997; Toussaint 1999; Wallis and Joubert 1972). The second type of symptom consists of small black spots scattered along the leaf surface (Sahin and Miller 1997).

Epidemiology

Research on the epidemiology of this organism is limited. The original outbreak in 1918 was thought to have originated from infested seed in commercial seed lots (Ohata *et al.* 1982). However, the organism went undetected when these commercial seed lots were tested. Other studies have subsequently demonstrated that the bacterium can be transmitted by infected seed (Ohata *et al.* 1982; Pernezny *et al.* 2001; Umesh *et al.* 1996). Wellman-Desbians (1999) studied the ability of *X. c.* pv. *vitians* to spread in the greenhouse. Planting infected seeds resulted in infected plants which resulted in the spread of the pathogen throughout the greenhouse via overhead irrigation (Wellman-Desbians 1999). A few infected seed can lead to many diseased transplants and may mean that the pathogen can cause significant field epidemics when even low rates of seed transmission occur.

X. c. pv. *vitians* can survive in soil associated with plant debris (Barak *et al.* 2001). Survival of *X. c.* pv. *vitians* on crop debris and in soil was studied over time. It was found that high populations of *X. c.* pv. *vitians* were recoverable from field debris one month after fields had been plowed in the summer. BLS was also determined to over-summer and produce disease symptoms on the lettuce crop the next fall season.

X. c. pv. *vitians* has also been shown to survive on lettuce leaf surfaces as an epiphyte using standard dilution plating techniques (Toussaint 1999). Toussaint *et al.* (2001) used scanning electron microscopy to find bacteria on the leaf surface of asymptomatic leaves.

Information on the optimal temperature for development of BLS has been inconsistent. *X. c.* pv. *vitians* was originally reported by Brown (1918) to grow optimally between 26 and 28º C. Later reports (Toussaint 1999; Pernezny *et al*. 1995) observed

that disease development occurred in warm moist conditions. Zoina and Volpe (1992) and Brown (1918) observed that BLS occurred after a drop in temperature and a frost. Patterson *et al.* (1964) observed that disease development occurred after a drop in temperature, but also states that BLS arises in the warm lettuce growing areas of California.

There have been a number of studies on the host range of *X. c.* pv. *vitians* within crop species as well as weed species. Tsuchiya *et al.* (1981) performed experiments on 99 crop species from 19 families as well as 97 weed species from 31 families in Japan in these studies, plants were artificially inoculated with the pathogen and rated for disease incidence. They found a number of hosts within *Cruciferae*, *Polygonaceae*, *Tropaeolaceae*,and *Compositae*. Sahin *et al*. (1997) studied eight commercial lettuce cultivars. They found high susceptibility in the cos as well as two of the green leaf types. Carisse *et al.* (2000) reported that the most susceptible cultivars were butterhead and cos types with the least susceptible being green leaf types in Canada. Pernezny *et al.* (1995) found that the cos types were the most susceptible in Florida. Although a plethora of host range studies have been conducted. There is no information has been presented on the internal or external population dynamics of *X. c.* pv. *vitians*. All reports have used lesion development and disease characteristics to determine host range. Determination of epiphytic and *in vivo* populations would contribute to knowledge on the host range of *X. c.* pv. *vitians*.

Little information is available on the effect of wounding on host range. Tsuchiya et al. (1981) used needles to artificially inoculate then sprayed with a suspension diluted to

10^8 cells/µl. Frequently during the rainy season heavy downpours and blowing sand occur. This may increase the chances for infection.

The objectives of this study were

1. To determine the optimum temperature for disease development of the *X. c.* pv. *vitians*.
2. To determine if wounding affects the onset and severity of disease on different potential hosts.
3. To ascertain the population dynamics of *X. c.* pv. *vitians* within the leaf tissue of several putative hosts.
4. Evaluate epiphytic populations of *X. c.* pv. *vitians* on lettuce and other salad crops currently grown commercially in the Everglades farming region.

CHAPTER 2
OPTIMUM TEMPERATURE FOR DEVELOPMENT OF BACTERIAL LEAF SPOT OF LETTUCE

In the winter of 1992-1993, a damaging outbreak of bacterial leaf spot (BLS) of lettuce occurred in the Everglades Agricultural Area (EAA) of South Florida (Pernezny *et al.* 1995). In the 1990's, BLS caused by *Xanthomonas campestris* pv. *vitians* was reported from around the world (Daboin and Tortolero 1991; Sahin 2000; Sahin and Miller 1997; Toussaint 1999; Tsuchiya et al. 1981; Wallis and Joubert 1972; Zoina and Volpe 1992). While BLS generally does not reduce plant size it does render lettuce unmarketable due to reduced quality associated with unsightly blemishes.

Research has been published on the epidemiology of this organism (Barak et al. 2001; Pennisi and Pane 1990; Toussaint et al. 2001; Tsuchiya et al. 1981; Wellman-Desbians 1999). Comparisons of these reports show an inconsistency in the description of temperatures and weather conditions favoring the epidemics. When the disease was first described, Brown (1918) suggested the optimum growth temperature of the organism to be between 26º and 28º C. The outbreak in which Brown obtained her strains occurred after a sudden drop in temperature in February in South Carolina. Patterson described the development of BLS as occurring in warm lettuce growing areas (Patterson et al.1986). In the Salinas valley of California, an epidemic occurred in April when the daily temperatures averages16.1º C (3º F) below normal. The average temperature in the Salinas Valley is 14º C in April (Anonymous 1990). Toussaint reports the optimal *in vitro* temperature to be 28ºC and observed the epidemic under warm, humid, and rainy

conditions for Canada (Toussaint 1999). The outbreak was observed in Canada during July and August, which has average temperatures in the teens and highs near 30ºC (Agriculture 2003; Vose *et al.* 2003). Pernezny reported the 1992 epidemic to have occurred during the winter in the Everglades Agricultural Area which was unusually warm (Pernezny *et al.* 1995). In Italy, the epidemic occurred after an unusual cold spell in November, with accumulated frost (Zoina and Volpe 1992). Southern Italy has an average November temperature of 12.5º C (Anonymous 1990). The contradictions in the most recent reports warranted an investigation of the influence of temperature on disease development.

The goal of this study was to determine the optimum temperature for the development of BLS on lettuce plants under controlled growth chamber conditions.

Materials And Methods

Growth of Plants

Cos (cv. Valmaine) lettuce seeds were germinated in 10-cm-diameter pots in a commercial soil mix Farfard no. 2 (Conrad Farfard, Inc., Agawan, MA.). Every two weeks, each pot received 25 ml. of a soluble fertilizer and 25ml of a slow release fertilizer (Scotts, Marysville OH). The experiment was maintained in an air-conditioned greenhouse with temperatures ranging from 23º to 27º C. Plants were moved to a conviron (Controlled Environments Inc, Pembina N.D.) growth chamber at 4 and 6 weeks of age. Growth chambers were set at four different temperatures: 15, 20, 25, and 30º C.

Preparation of Inocula and Inoculation

X. c. pv. *vitians* strain L41, isolated near Belle Glade, FL in 1998, was used in these tests. The pathogen was grown for 3 days at 28º C on nutrient agar (NA) plates. Plates

were flooded with sterile tap water and suspensions were adjusted turbidimetrically to ca. $1x10^8$ colony forming units (CFU) /ml using a spectrophotometer. Suspensions were diluted to $1x10^7$ CFU/ml. Five plants, each between 4 to 6 weeks old were sprayed with the bacterial suspension until run-off on both the adaxial and abaxial surfaces using a hand-held pump sprayer. Controls consisted of two lettuce plants sprayed with sterile tap water and placed at each temperature. All plants were immediately encased in clear plastic bags. After 3 days, bags were removed and plants arranged in a completely random design in the growth chamber.

Evaluation of Disease

Twelve days after inoculation, plants were evaluated for disease. Lesions were counted on all leaves and data recorded per symptomatic leaf. Lesion counts were expressed as lesions per plant.

Statistical Methods

The "best fit" equation relating disease severity (expressed as number of lesions) to temperature was determined by regression using The GLM Procedure of SAS (SAS Inc. Cary N.C.). The quadratic relationship, providing the best fit of data was used to determine the optimum temperature for disease development by setting the first derivative of the regression curve to zero and solving this equation for the x value (temperature). This corresponded to the inflection point of the regression curve (slope=0).

Results

Differences in the appearance and severity of disease were observed and quantified as lesions found per symptomatic leaf per plant as a function temperature (Fig. 2-1). Consistently low lesion counts per plant were observed at 15º C throughout three trials

(Fig. 2-2.) The highest lesion counts in the trials occurred at 25º C. A regression equation was derived from the plot of number of lesions vs. temperature. From the equation for the regression, the temperature optimum was calculated as described above in statistical methods. The equation for the regression was y = -5695.29+588.2x-12.904x2 where y equals the number of lesions and x equals temperature. The y-intercept according to the GLM procedure output was -5695.29 where $p \leq 0.038$. The R^2 value equaled 0.328876. The temperature for optimal disease development according to the model was found to be 22.7ºC. See figure 2-1.

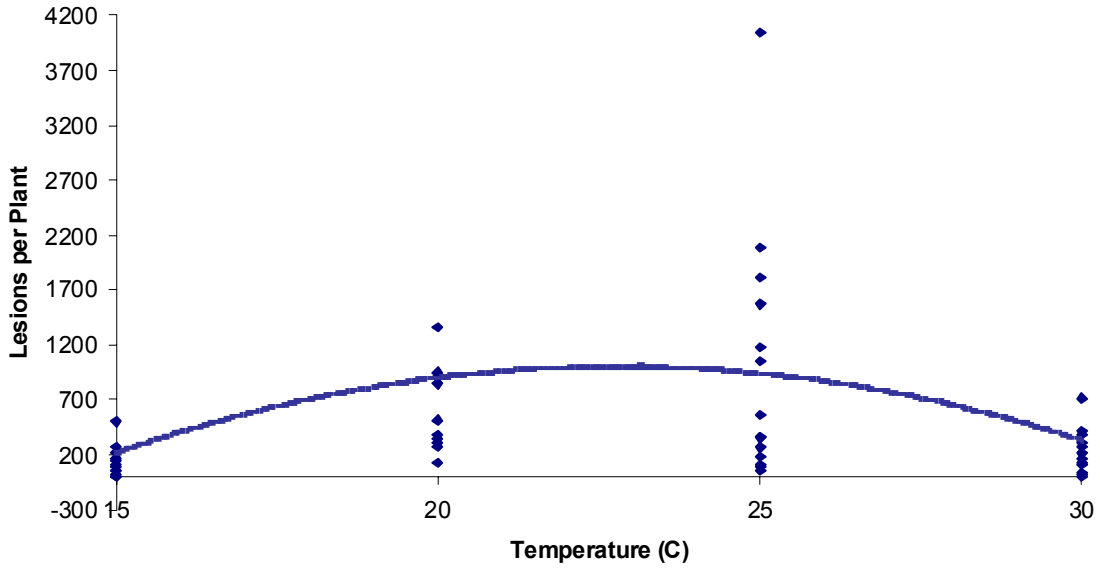

Figure 2-1. The effect of incubation temperatures on BLS disease development on lettuce plants inoculated with *X. c.* pv. vitians. Scatterplot of regression (y= -5695.29 + 588.2x – 12.90x2) with individual data points from three trials describing lesions per plant twelve days after inoculation.

Figure 2-2. The effect of temperature on the development of bacterial leaf spot. Plants were placed at 15, 20, 25, and 30ºC, lettered A, B, C, and D, respectively. Symptoms were noticeably more severe on plants placed at 25ºC.

Discussion

The literature is inconsistent in its description of the optimum temperature for disease development. Brown (1918) determined the optimum temperature for growth of *X. c.* pv. *vitians* to be between 26º and 28º C. However, over time the literature has reported the optimal temperature for disease development of BLS to be in a warm, moist (Toussaint 1999; Pernezny *et al*.1995) climate and a cool climate (Zoina and Volpe 1992). The type of experiment and equipment used by Brown is unknown and unpublished. Studying strains from the Everglades Agricultural Area of Florida, it was found that the optimal temperature for disease development was 22.7º C, a relatively cool temperature. The organism tends to infect at cooler times of the year in South Florida.

Pernezny first reported an epidemic of BLS in the winter of 1992-1993 (Pernezny *et al.* 1995). Based on weather data from that time period states the average temperature use 18.7º C in December and 20º C in January (Everglades 2003). These temperatures are slightly lower than the model derived from the study. However, the average high temperatures go well into the upper 20ºs.

Although temperature requirements are important for the epidemiology of BLS, the amount of rainfall and relative humidity levels also play significant roles in the induction of disease (Agrios 1997). Many plant diseases do not become epidemic, even if the susceptible host plant and the virulent pathogen are present. Growers in Florida should be aware that BLS occurs in relatively cool temperatures and is more likely to cause problems during the cooler months in Florida or when there is an unusual cold spell. However, other factors may also be important. An increase in moisture may be one factor which increases the chances of epidemic occur. The winter of 1992-1993 in the EAA was one in which the rainfall was much above average. The average rainfall for EAA is 58.1 mm in January, but that year 258 mm fell (Everglades 2003). Also cultural practices could increase injury to plants and increase the chances of an epidemic. Most lettuce is direct-seeded in Florida, which means that manual thinning must be performed. This thinning may injure neighboring plants creating an excellent avenue for pathogen ingress (Pohronezny *et al.* 1990). The knowledge of the optimum temperature for disease development of BLS as well as other factors which are known to incite epidemics can assist growers in determining when an epidemic may occur.

CHAPTER 3
POPULATION DYNAMICS OF *Xanthomonas campestris* pv. *vitians* IN LETTUCE AND OTHER SALAD CROPS

Xanthomonas campestris pv. *vitians*, caused a significant epidemic of bacterial leaf spot (BLS) of lettuce in the winter of 1992-1993 in the Everglades Agricultural Area (EAA) of South Florida (Pernezny *et al.* 1995). It has also caused serious problems in other lettuce growing regions throughout the world (Boeswinkel 1977; Harrison 1963; Pennisi and Pane 1990; Sahin 2000; Toussaint 1999; Wallis and Joubert 1972). Brown, water-soaked lesions are typically found on the crop. BLS generally does not produce a large crop loss; however, the damaged appearance of the lettuce makes it unmarketable.

An increase in the pre-washed, bagged lettuce market has changed the current cropping system in the EAA to one in which a number of closely related plant species are planted in close proximity to lettuce. The convenience of growing several crop species near to one another increases the possibility that one salad crop species, such as cilantro, could harbor *X. c.* pv. *vitians*, yet not produce typical disease symptoms or remain symptomless. In one study, it was shown that a symptomless weed, *Leersia hexandra (SW.)*, which grows adjacent to rice fields in Texas, supported large internal populations of *Xanthomonas campestris* pv. *oryzae* (4.0×10^5 CFU/g) over a twenty two day period (Gonzalez *et al*. 1991). It is possible that external populations of bacteria can exude from internal populations as it does with citrus canker (Timmer *et al.* 1991) or may survive on alternative, more resistant hosts until weather conditions change and lettuce becomes susceptible to infection by the pathogen which occurs with wildfire in tobacco (Diachun

and Troutman 1946). Determining which plant species may harbor high populations of *X. c.* pv. *vitians* would be useful information for the growers of South Florida. The host range of *X. c.* pv. *vitians* within cultivars of lettuce, other crop species and some weed species has been described (Carisse *et al.* 2000; Pernezny *et al.* 1995; Sahin *et al.* 1997; Tsuchiya *et al.* 1981). In previous studies plants were assessed for disease severity. No studies have been published on the population dynamics of this bacterium following infiltration of the leaves with the bacterium. Information on the internal population dynamics in different plant species could help determine which are potential hosts and therefore reservoirs for disease.

The objective of this study was to determine the population dynamics of *X. c.* pv. *vitians* within leaf tissue of various plant species.

Materials and Methods

Plant Culture

Seeds from each plant species were germinated in 10-cm-diameter pots in a commercial soil mix Farfard no. 2 (Conrad Farfard, Inc., Agawan, MA.). Every 2 weeks, each pot received 25 g of a soluble fertilizer (Scott's Co., Marysville. OH). Pots were maintained in an air-conditioned greenhouse with temperatures ranging from 18.3º to 25.5ºC.

Preparation of Inoculum

The L7R strain of *X. c.* pv. *vitians*, a one-step spontaneous mutant derived from L7 and resistant to 100 μg/ml rifampicin. The original L7 strain was isolated from diseased lettuce in the EAA, was used in all experiments. L7R was grown for 3 days at 28ºC on nutrient agar (NA) plates. The plates were flooded with sterile buffered saline (Leben *et al.* 1968) and suspensions were adjusted turbidimetrically to ca. $1x10^8$ CFU/ml using a

spectrophotometer. Suspensions were diluted to $1x10^5$ CFU/ml in buffered saline. Five leaves of each potential host, on 4-5 wk. old plants, were injected with the suspension using a hypodermic syringe without a needle. Controls consisted of two plants infiltrated with sterile buffered saline solution. Periodically over a 14 day period, leaves were collected and surface sterilized by dipping in a 10% sodium hypochlorite solution followed by a rinse in de-ionized water. Leaves were blotted until dry on sterile paper towels. A 1-cm^2 area of leaf tissue was cut out and ground in 2 ml of buffered saline solution, serially diluted, and plated on nutrient agar plates amended with rifampicin. Populations were determined 1, 3, 5, 8, 11 and 14 days after inoculation (DAI). Populations were calculated based on CFU/cm^2 fresh tissue.

Results

Internal populations of *X. c.* pv. *vitians* developed higher peak populations in lettuce than in the other plant species tested (Fig. 3-1). In trial 1, lettuce populations reached a maximum of 3.5×10^8 CFU/cm^2 at 8 DAI. The second highest populations occurred in pepper which reached peak populations of 5.8×10^6 CFU/cm^2 at 10 DAI. Tomato and cilantro had generally lower population levels with a definite decrease at 10 DAI. Populations in parsley rose early on in the experiment, until 3 DAI when they decreased for most of the experiment. Populations in beet started at low levels until 10 DAI when populations inexplicably rose to 2.0×10^5 CFU/cm^2 on 14 DAI.

In trial 2, internal populations of *X. c.* pv. *vitians* followed similar trends (Fig. 3-2). Again, lettuce had significantly higher populations of *X. c.* pv. *vitians* with the maximum being 1.1×10^8 log CFU/cm^2 on 5 DAI. Populations of *X. c*. pv. *vitians* throughout were second highest in pepper. Populations reached 3.8×10^5 CFU/ cm^2 in tomato. Parsley

populations reached 3.5 x 10^6 CFU/cm^2. Populations in cilantro never went above 1.3 x 10^6CFU/ cm^2, whereas populations remained about 10^3 CFU/ cm^2 in beet.

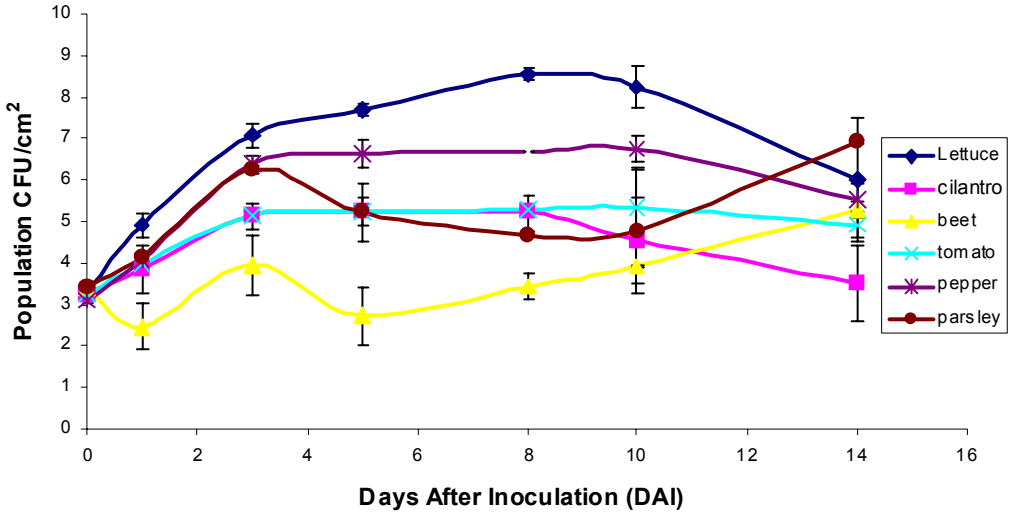

Figure 3-1. Bacterial populations in selected plant species from Trial 1following with 10^5 CFU/ ml. *X. c.* pv. *vitians*.

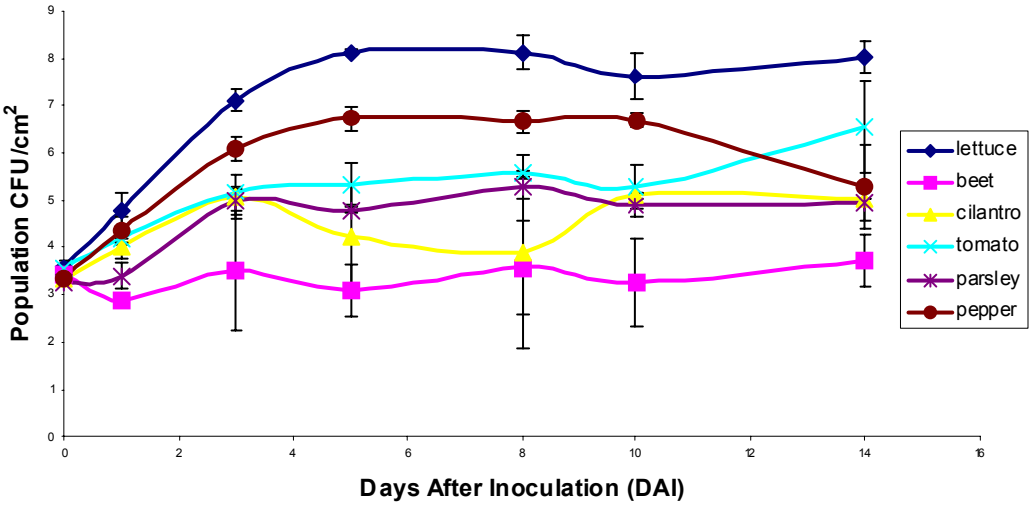

Figure 3-2. Bacterial populations in selected plant species from Trial 2 after infiltration with 10^5 CFU/ml *X. c.* pv. *vitians.*

Symptoms varied depending on the plant species. By 14 DAI, lesions were brown and papery in lettuce. Symptoms in pepper and tomato were beginning to brown, but were noticeably less numerous. The other inoculated plant species were asymptomatic.

Discussion

Lettuce supported higher populations of *X. c.* pv. *vitians in vivo* than the other plant species in this test. This is not surprising since lettuce is a natural host. Bacterial populations in lettuce grew to levels of 10^8 CFU/cm^2 and showed typical disease symptoms. Populations in pepper reached a log unit lower than lettuce with appearance of symptoms which suggests that it may be an additional host. Sahin & Miller (1997) reported pepper and tomato as hosts, but no population levels were measured *in vivo*. The appearance of lesions and the fairly high *in vivo* populations attained in this study lends support to the conclusion of Sahin & Miller that pepper and tomato may be alternative hosts.

Identification of plant species which support high populations of *X. c.* pv. *vitians* facilitates decisions regarding future crop rotations in lettuce growing regions of the world. Knowing that these two plant species can harbor such high populations, a crop rotation in which pepper and tomato are not planted in the same area as lettuce may be a good idea for prevention of spread of *X. c.* pv. *vitians*. Beet, cilantro, and parsley did not support populations at levels as high as lettuce, pepper and tomato. Under field conditions, these plant species would probably not be a good source of inoculum for other susceptible plant species and could be planted in close proximity to lettuce.

Under normal growing conditions, the risk of an epidemic originating in tomato or pepper would probably be quite low because populations may not reach levels high enough to disperse from tomato or pepper and infect adjacent lettuce. In the EAA,

consisting primarily of histosols, this would be especially true because growers do not plant tomato and pepper on the muck soils due to the high nitrogen content. Overall, tomato and pepper do not pose much of a risk to EAA growers, but it is important to note the high population levels supported for future epidemiological studies with *X. c.* pv. *vitians*.

CHAPTER 4
SURVIVAL OF EPIPHYTIC *Xanthomonas campestris pv. vitians* POPULATIONS IN THE FIELD

Xanthomonas campestris pv. *vitians*, the causal organism of bacterial leaf spot (BLS) on lettuce, caused a severe epidemic in the winter of 1992-1993 in the EAA area of Florida (Pernezny *et al.* 1995). The brown, water-soaked lesions caused by *X. c.* pv. *vitians* generally do not reduce crop biomass, but instead make the crop unmarketable due to its appearance (Pernezny *et al*. 1995). BLS appears to be a significant problem worldwide (Daboin and Tortolero 1991; Pennisi and Pane 1990; Sahin 2000; Toussaint 1999). It was thought that the pathogen was disseminated on infested seed, but when seed lots were assayed for *X. c.* pv. *vitians*, the bacterium was not detected (Ohata *et al.* 1982).

X. c. pv. *vitians* has been shown to survive and be transmitted to seedlings from infested seed (Ohata *et al.* 1982). The bacterium also has been shown to be disseminated in irrigation water within greenhouses (Wellman-Desbians 1999).

Epiphytic bacteria are considered to be the bacterial communities which exist on the leaf surface which can be removed by washing (Hirano and Upper 1983). In the epiphytic phase, bacteria survive on leaf surfaces without infecting the plant. The epiphytic phase can be a survival stage in the life cycle of bacteria (Swings and Civerolo 1993). Mew and Kennedy (1971) determined that host susceptibility determined epiphytic population size. They observed that *Pseudomonas glycinea* increased and diminished more quickly on cultivars of soybean with intermediate susceptibility

compared to highly susceptible cultivars on which populations continued to grow steadily. Bacterial pathogens can survive on a plant species as epiphytes without producing symptoms and later serve as inoculum for nearby hosts (Leben, 1981). *Pseudomonas syringae* pv. *syringae*, causal organism of bacterial brown spot of bean survived among the epiphytic flora on hairy vetch weeds next to bean plants (Ercolani *et al.* 1974). When conditions were conducive for the spread of the pathogen, such as an increase in rainfall, the epiphytic bacteria were shown to move from symptom-less hairy vetch leaves to bean fields. In several cases, epiphytic populations of plant-pathogenic bacteria are known to exist from several days to months before resulting in disease (Weller and Saettler 1980; Digat 1978; Zoina and Volpe 1992). It has also been shown that a correlation exists between the presence of high epiphytic populations on symptomless weed species and outbreaks of disease (Ercolani *et al.* 1974; Rouse *et al.* 1985).

Agricultural practices in south Florida have been changing in recent years. The salad crop production area is being reduced because the nature of the industry is changing in response to the pre-mixed, bagged salad market. It is now a common practice to plant long rows of different plant species in the same field. This integration of salad crop species on commercial farms could increase the importance of epiphytic populations of bacterial pathogens on non-host plants.

There is little published information on *X. c.* pv. *vitians* epiphytic survival on different crop species. Since current cropping patterns in the EEA routinely involve growing several crops next to one another, these studies were initiated to determine the

epiphytic populations on crops commonly grown in close proximity to lettuce in South Florida.

Materials and Methods

Bacterial Strain and Culture

L7R, a rifampicin resistant strain derived from L7, originally recovered in 1995 from the Everglades area and resistant to 100µg/ml rifampicin, was used for all inoculations. L7R was grown for 3 days at 28º C on nutrient agar (NA) plates. These plates were flooded with sterile buffered saline (Leben *et al.* 1968) and suspensions were adjusted turbidimetrically to ca. $1x10^8$ CFU/ ml using a spectrophotometer.

Field Study

Six different species of salad crops were transplanted onto raised beds on 90-cm centers in a randomized complete block design at the Pine Acres Research Farm in Marion County, Florida on 6 March 2003. The beds were covered with plastic mulch and fumigated with methyl bromide to reduce weed, insect, and soil-borne pathogen populations two weeks prior to planting. The plastic mulch was removed prior to transplanting for the remainder of the growing season. Plants were fertilized using a 6-8-6 liquid fertilizer delivered season long through a drip system. Four to five week old transplants were spray-inoculated with 10^8 CFU/ml using a backpack sprayer. Plants were sprayed to runoff in the evening at approximately 18:00 hr.

Immediately following inoculation (week 0 after inoculation WAI), approximately 1 g of leaf tissue was harvested from each treatment in two replicates of the experiment to establish a baseline populations of *X. c.* pv. *vitians*. At the same time, plots were rated for disease using a 1 to 100 scale of disease severity (Table 4-2). These sampling procedures were repeated every week for the next 5 weeks. All samples were from

asymptomatic leaves. Leaf samples were immediately placed in plastic bags in a cooler and transported to the laboratory. Fresh weights of sample were determined, and tissue placed in 250 ml Erlenmeyer flasks containing 10 ml of sterilized tap water per gram of tissue. Flasks were shaken on a wrist action shaker for 30 min. The leaf washings were serially diluted and plated on NA plates amended with rifampicin. The plates were incubated in a 28ºC growth chamber for 72 h. The colonies (30-300 colonies/ plate) were counted 72 h later and expressed as Colony Forming Units CFU/ gram tissue. The data were analyzed using a SAS PROC GLM followed by the mean separation procedure, LS MEANS ($p \leq 0.05$) (SAS Inc, Cary N.C.).

Results

The growing season populations of *X. c.* pv. *vitians* remained high at 10^4-10^5 CFU/gram on lettuce until the fifth week when populations dropped dramatically (Figure 4-1). Populations of *X. c.* pv. *vitians* on tomato followed a curve similar to that of lettuce with populations often only 1 or 2 log units different than those associated with lettuce. Populations on all crops were very low (10^1 CFU/ g to undetectable) at the end of the experiment. Populations of *X. c*. pv. *vitians* remained statistically higher than beet, cilantro, and parsley at 2 WAI. At 3 WAI all plant species supported statistically similar populations (Log 0-1) (Table 4-1).

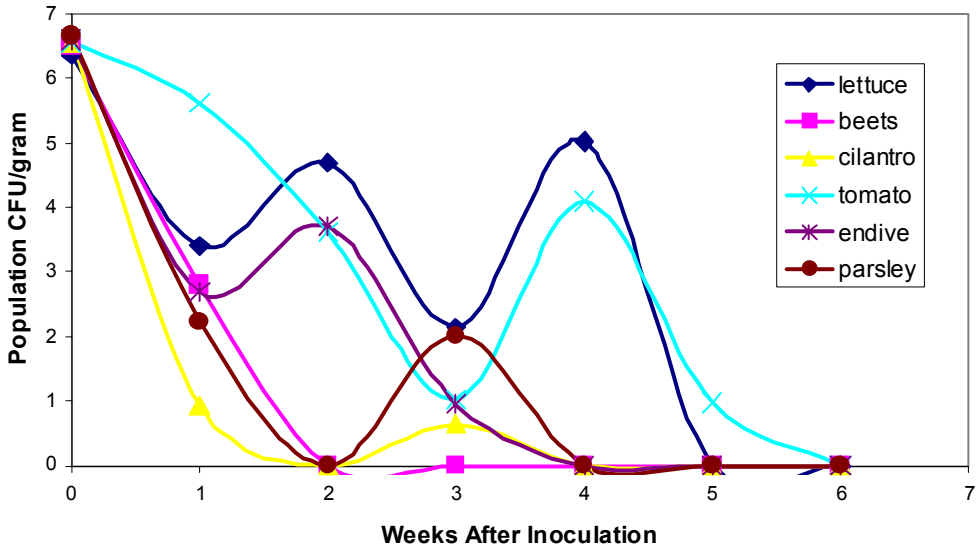

Figure 4-1. Epiphytic populations of *X. c.* pv. *vitians* on lettuce, beets, cilantro, tomato, endive, and parsley over a five week period in Spring 2003.

Table 4-1. Population dynamics of *X. c.* pv. *vitians* on asymptomatic leaves over time on six plant species.

	Sample Period[a]				
Species	Week 1	Week 2	Week 3	Week 4	Week 5
Cilantro	0.9 B[b]	0.0 B	0.6 A	0.0 C	0.0 A
Parsley	2.3 B	0.0 B	2.0 A	0.0 C	0.0 A
Beets	2.8 AB	0.0 B	0.0 A	0.0 C	0.0 A
Tomato	5.6 A	3.6 A	1.0 A	4.1 B	1.0 A
Endive	2.7 AB	3.7 A	0.9 A	0.0 C	0.0 A
Lettuce	3.4 AB	4.7 A	2.1 A	5.0 A	0.0 A

[a]Mean population ($\log_{10}$ CFU/g)

[b]Numbers in columns followed by the same letter are not significantly different at $P<0.05$ according to the LS Means test.

Discussion

Our results demonstrate the ability of *X. c.* pv. *vitians* to multiply epiphytically on several crops which might be planted in close proximity to one another in some agricultural systems. The use of this field plot design simulates natural environmental conditions in which these crops are grown. The presence of epiphytic populations on the different crops allows us to infer the population dynamics of this pathogen that may occur in commercial fields.

Table 4-2. Percent foliage infected by *X. c.* pv. *vitians* in field plots over a four week time period.

		DISEASE SEVERITY (% Foliage Infected)			
PLANT	REP	WEEK 2	WEEK 3	WEEK 4	WEEK 5
Lettuce	1	2	5	10	7
	2	10	10	15	5
	3	2	10	15	5
	4	2	2	5	2
Parsley	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
Cilantro	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
Tomato	1	20	25	20	12
	2	20	10	20	15
	3	2	15	15	7
	4	2	2	2	1
Endive	1	0	0	0	0
	2	0	0	0	0
	3	1	2	0	0
	4	0	0	0	0
Beet	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0

Epiphytic populations are in a continual dynamic. Variation in epiphytic populations from leaf to leaf on the same plant species is considerable. Population sizes may vary by at least 10-fold even on leaves of the same size and age (Hirano and Upper 1983, Hirano 1990). Even when plants have been grown in similar environmental conditions, it has been shown that different plant species support very different populations of epiphytic bacteria (Lindow *et al.* 1978; O'Brien and Lindow 1989). The factors that affect the development and growth of epiphytic bacterial populations are still under scrutiny. Among other things available moisture, UV radiation, nutrients, and plant species are all factors which can be involved (Mercier and Lindow 2000; O'Brien

and Lindow 1989). No rainfall occurred during the course of this experiment, which probably resulted in an environment less likely to favor the growth and multiplication of epiphytic bacteria. Since populations were still found on lettuce and tomato under low moisture conditions at 4 WAI it may be that these two crops may be a source of inoculum if environmental conditions changed in favor of development of BLS. It would be prudent for Florida growers to not plant endive near to lettuce, a susceptible host during the winter months of Florida when weather conditions may be conducive to the growth of BLS. Results indicate that epiphytic populations did not survive on beet, cilantro and parsley over time. These plant species would be good choices for the farmers in South Florida to use in contiguous plantings or in rotation with lettuce.

CHAPTER 5
THE EFFECT OF WOUNDING ON THE DEVELOPMENT OF BACTERIAL LEAF SPOT ON LETTUCE

In the winter of 1992-1993 bacterial leaf spot of lettuce (BLS) was first identified in the Everglades Agricultural Area (EAA) and caused serious damage to the lettuce crop (Pernezny et al. 1995). Within the same few years, the pathogen, *X. c.* pv. *vitians,* was shown to cause outbreaks in other states in the U.S. as well as throughout the world (Daboin and Tortolero 1991; Pennisi and Pane 1990; Sahin 2000; Sahin and Miller 1997; Toussaint 1999; Tsuchiya *et al.* 1981; Wallis and Joubert 1972). *X. c.* pv. *vitians* is a gram negative rod, which produces brown water-soaked lesions. The appearance of Bacterial Leaf Spot (BLS) in lettuce renders the crop unmarketable. Initially it was thought that *X. c.* pv. *vitians* was introduced by infested seed (Ohata *et al.* 1982). However, attempts to isolate the pathogen from commercial seed lots failed. Aspects of the etiology and epidemiology of BLS remain to be explored.

Commercial lettuce cultivars have been studied for their susceptibility to BLS. Butterhead, cos and two green, leafy types are highly susceptible (Carisse *et al.* 2000; Pernezny *et al.* 1995). Host range studies with *X. c.* pv. *vitians* within lettuce cultivars and other crop species as well as weed species have been performed (Tsuchiya *et al*. 1981). Tsuchiya artificially inoculated and rated for disease incidence (Tsuchiya *et al.* 1981). For a number of species within the *Cucurbitaceae*, *Cruciferae*, *Polygonaceae*, *Tropaeolaceae*,and *Compositae* showed symptoms. *X. c.* pv. *vitians* caused disease in wounded and non-wounded plants. Only closely related plants in the *Asteraceae* families

appear to be susceptible according to Pernezny and Raid (unpublished data). Sahin & Miller (1997) identified tomato and pepper as possible hosts.

Wounding predisposes numerous plants to increased disease severity (Johnson and Miliczky 1993; Pohronezny *et al.* 1992; Vakili 1967). Little is known about the effect of the wounding under simulated field conditions on BLS incidence and severity.

Materials and Methods

Plant Culture

Seeds of salad crop plant species listed in Table 5-1 and 5-2 were germinated in a commercial soil less mix Farfard no. 2 (Conrad Farfard, Inc., Agawan, MA.) 10-cm diameter pots and maintained in an air-conditioned greenhouse with maximum temperatures between 18.3º to 25º C. Every two weeks, each pot received 25ml of a soluble fertilizer (The Scotts company, Marysville, OH).

Inoculum Production

X. c. pv. *vitians* strain L7R, a strain resistant to 100µg/ml derived from L7 and originally isolated in 1995 from the Everglades Agricultural Area (EAA), was grown for 3 days at 28ºC on nutrient agar (NA) plates. These plates were flooded with sterile buffered saline (Leben *et al.* 1968) and suspensions were adjusted turbidimetrically to ca. $1x10^8$ CFU/ ml using a spectrophotometer. Suspensions were diluted to $1x10^7$ CFU/ml and used as inocula.

Inoculation Procedure

Five plants of each plant species that were about 4-5 weeks old, were sprayed inoculated with the bacterial suspension with or without wounding with the bacterial suspension to run-off on both the adaxial and abaxial surfaces using a hand-held pump sprayer. Wounding was accomplished by casting fine sand particles at the abaxial

surfaces of the leaves to simulate wounding resulting from wind-blown sand. Controls consisted of two wounded and two non-wounded plants of each test species sprayed with sterile buffered saline and two additional plants untreated in any way. All plants were immediately placed in clear plastic bags. After four days, the bags were removed and the plants were arranged in a completely randomized design on the greenhouse bench. Plants were observed for disease development at 11 and 16 days after inoculation (DAI).

Statistical Methods

Data were analyzed by ANOVA using the PROC GLM ($P \leq 0.05$) procedure in SAS (SAS inc. Cary N.C.) software. Means in an array were separated by Duncan's mean separation test at $P \leq 0.05$.

Results

In Trial 1 most plant species were unaffected by wounding. However, *Brassica oleracea* var. *capita* (cabbage) and *Brassica rapa* sp. *chinensis* (tatsoi) both had a marked increase in disease development in the non-wounded plants (Table 5-1). Lower levels of disease occurred in lettuce, but non-wounded plants showed more disease. In trial 2, only lettuce expressed disease symptoms with the wounded plants having slightly higher disease severity than non-wounded. (Table 5-2).

Table 5-1. Average disease severity ratings of wounded and non-wounded plants in Trial 1.

Plants	Wounded	Non-Wounded
Asteraceae		
Coriandrum sativum	0	0
Cichorum intybus 'Fiero F1'	0	0
Cichorum intybus 'Jupiter F1'	0	0
Cichorum endivia 'Lorca'	0	0
Lactuca sativa 'SX405'	0.33	2.3
Apiaceae		
Anethum graveolens 'Mammoth'	0	0
Daucus carota subsp. *sativas* '7099'	0	0
Petroselinum crispum 'Jade'	0	0
Brassicaceae		
Brassica juncea	0	0
Brassica oleracea var. capita	0	12.4
Brassica rapa sp. *chinensis*	0	0.4
Chenopodiaceae		
Beta vulgaris 'Darko'	0	0
Spinacia oleracea 'seminis'	0	0
Cucubitaceae		
Citrullus vulgaris 'crimson sweet'	0	0
Crucifereae		
Eruca vesicaria var. *sativa*	0	0
Tropaeolaceae		
Nasturtium officinale 'true water'	0	0

Ratings based on percent foliage affected.

Discussion

X. c. pv. *vitians* can cause symptoms on lettuce when inoculated with a bacterial suspension in an environment conducive to bacterial growth (Pernezny *et al.* 1995; Toussaint *et al.* 2001). Wounding predisposition is not required. However, *X. c*. pv. *vitians*, like most plant bacterial pathogens, does not have the ability to produce enzymes which degrade plant cell walls. Therefore, they must have an opening to enter and cause disease. The pathogen can enter the susceptible plant through wounds as well as and natural openings such as stomates (Agrios 1997). In these experiments disease

development was insufficient to draw conclusions about the relationship between wounding and the subsequent severity of BLS on lettuce. Vakili (1967) and Pohronezny *et al.* (1992) showed marked increases in the severity of bacterial spot on tomato and pepper, respectively, when plants were wounded just prior to arrival of the pathogen on plant surfaces. Unfortunately, it may be that the lack of sprinkler irrigation of test lettuce plants in growth chambers precluded normal disease development. Pernezny *et al*. (personal communication) achieved optimum BLS development in the greenhouse when foliage was overhead-irrigated daily.

Table 5-2. Disease severity ratings of wounded and non-wounded plants in Trial 2.

Plants	Wounded	Non-Wounded
Asteraceae		
Coriandrum sativum	0	0
Cichorum intybus 'Fiero F1'	0	0
Cichorum intybus 'Jupiter F1'	0	0
Cichorum endivia 'Lorca'	0	0
Lactuca sativa 'SX405'	2.3	0.67
Apiaceae		
Anethum graveolens 'Mammoth'	0	0
Daucus carota subsp. *sativas* '7099'	0	0
Petroselinum crispum 'Jade'	0	0
Brassicaceae		
Brassica juncea	0	0
Brassica oleracea var. capita	0	0
Brassica rapa sp. *chinensis*	0	0
Chenopodiaceae		
Beta vulgaris 'Darko'	0	0
Spinacia oleracea 'seminis'	0	0
Cucubitaceae		
Citrullus vulgaris 'crimson sweet'	0	0
Crucifereae		
Eruca vesicaria var. *sativa*	0	0
Tropaeolaceae		
Nasturtium officinale 'true water'	0	0

Ratings based on percent foliage affected.

CHAPTER 6
INFECTIVITY TITRATION AS THE BASIS FOR DETERMINATION OF HOST RANGE OF *Xanthomonas campestris pv. vitians*

In the winter of 1992-93, a major outbreak of bacterial leaf spot of lettuce, caused by *Xanthomonas campestris* pv. *vitians*, occurred for the first time in the Everglades Agricultural Area (EAA) of southern Florida (Pernezny *et al.*1995). The disease has occurred in several winter vegetable seasons in the EAA since then when precipitation has been higher than normal. It is possible that seedborne inoculum was responsible for these outbreaks (Carrisse, et al. 2000; Pernezny, *et al.* 2002; Sahin & Miller 1997).

Several studies have shown that there are clear differences in susceptibility of lettuce cultivars to bacterial leaf spot (Carrisse, *et al.* 2001; Pernezny, *et al.* 1995; Sahin & Miller 1997). Generally speaking, cos (romaine) lettuce types are more susceptible than crisphead (iceberg) or butterhead cultivars. These results, primarily from greenhouse experiments, correlate well with the apparent impact of this disease in commercial fields. Most of the serious damage in the EAA has occurred in plantings of cos lettuce.

The face of the vegetable industry in the EAA has changed significantly over the past 12 years. Concentrated, large acreages of crops, such as celery and lettuce, have been replaced by generally smaller plantings of a wider diversity of salad crops. These alternative crops include endive, escarole, radicchio, spinach, and several herbs (e.g. parsley and cilantro). Since lettuce and these other crops are now grown in close proximity both spatially and temporally, it was deemed prudent to determine the host range of *X. c.* pv. *vitians.* Preliminary experiments were carried out in the greenhouse

where the test plants of each species were spray-inoculated with a 10^7 CFU/ml suspension of the bacterium. No disease reaction was observed for arugula, celery, spinach, Swiss chard, carrot, dill, radicchio, chicory, mizuna, tatsoi, dandelion, beet, cabbage, or upland cress. However, disease reactions, consisting of water-soaked lesions that later turned necrotic, were observed for cilantro, endive, and escarole. Milder symptoms were observed in some of the parsley test plants. Disease ratings on the affected species were similar, and in some cases greater than those observed on the lettuce controls. Based on these results one could conclude that cilantro, endive, escarole, and possibly parsley might be subject to epidemics incited by *X. c.* pv. *vitians* in those seasons favorable for disease development. However, we have not observed the disease in any fields except lettuce, and at no time have growers brought samples of these other crops to our laboratory that suggested a bacterial leaf spot etiology. Further studies were deemed necessary to more fully explore the susceptibility of a number of these salad crops to *X. c.* pv. *vitians*.

Infectivity titration is one method that can be used to judge the relative susceptibility of genotypes to challenge from a potential pathogen (Ercolani et al.1984). In typical infectivity titration experiments, a range of concentrations of inoculum (e.g. 10^8, 10^7, 10^6...10^1 CFU/ml) are applied to sets of test plants. Observations are then made periodically of some measurable response in the hosts (e.g. appearance of leaf spots). As in its chemical titration counterpart, an end point may be sought. The end point used in many infectivity titrations is that concentration below which there is no longer visible evidence of the measured response (Ercolani et al.1984). The objectives of these studies were to establish the likelihood that the host range of *X. c.* pv. *vitians* includes more crop

species than lettuce based on quantal responses of test plants subjected to an infectivity titration series.

Materials and Methods

Seed of the following were planted in 10-cm-diameter plastic pots filled with Fafard Soil Mix No. 2 (Conrad Fafard, Inc., Agawan, MA): cos lettuce, cv. Valmaine; endive, cv. Markant; and parsley, cv. Moss Curled. Plants were grown in an air-conditioned greenhouse with a temperature range of 23-28ºC.

X. c. pv. *vitians* strain L7 (Pernezny *et al.* 1995) was grown on nutrient agar amended with 0.5% (w/v) glucose (GNA) amended with 50g/ml cycloheximide. Plates were flooded with phosphate-buffered saline (Pernezny *et al.* 1995), and a bent glass rod was used to suspend bacteria. Suspensions were adjusted turbidimetrically to approximately 2 x 108 CFU/ml. Five-week-old lettuce and endive and 8-week-old parsley plants were sprayed to run-off on both adaxial and abaxial surfaces between 0900 and 1130 hour. A decimal dilution series was used to adjust the original inoculum to concentrations of 10^{8}, 10^{7}, 10^{6}, 10^{5}, 10^{4}, 10^{3}, and 10^{2} CFU/ml. Each of the seven bacterial suspensions was then used to inoculate three replicates of each plant species. Plants sprayed with sterile buffered saline served as controls. Plants were immediately covered with clear plastic bags. After 4 days bags were removed. Disease assessments were made 13-14 days after inoculation. Two trials of this experiment were completed.

Results

Infectivity titration end points for lettuce, endive, and parsley were distinctly different. Good symptoms were observed in lettuce for four dilutions (10^{8} down to 10^{5} CFU/ml). Symptoms in endive, on the other hand, were not consistently evident below 10^{7} CFU/ml, except for one plant with mild symptoms at 10^{4}CFU/ml. In these two trials,

no symptoms were observed in parsley at any concentrations tested. Therefore, under the conditions of these experiments, the infectivity titration end points for lettuce, endive, and parsley are 10^5, 10^7, and 10^8 CFU/ml, respectively.

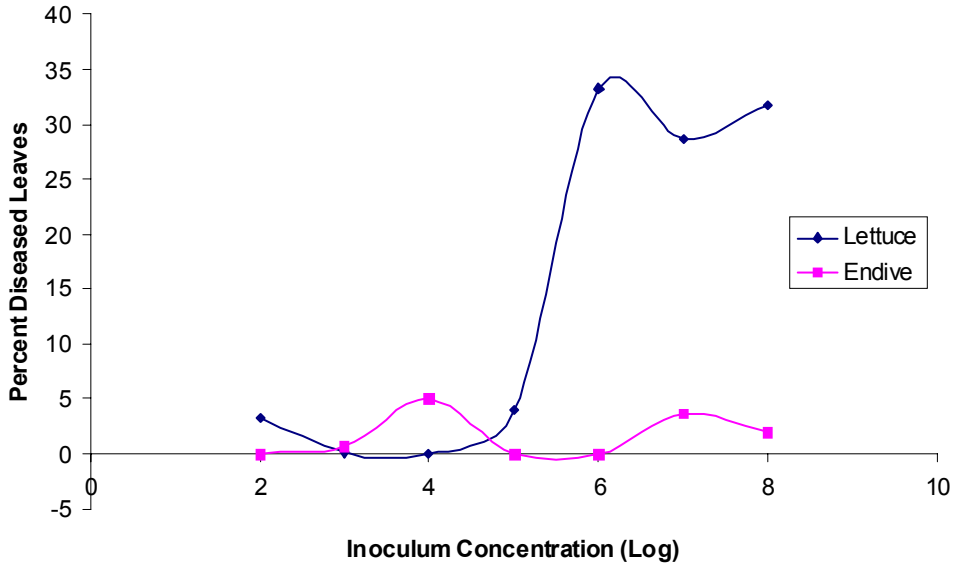

Figure 6-1. Concentration of X. c. pv. vitians effect on disease symptoms in lettuce and endive.

Discussion

Use of inoculum concentrations of 10^7 CFU/ml or higher may mislead observers studying the host range of *X. c.* pv. *vitians*. In preliminary tests, we inoculated a large number of salad and several herb crops with 10^7-10^8 CFU/ml suspensions of *X. c.* pv. *vitians*. In several cases, notably endive, escarole, and cilantro, we observed water-soaked leaf spots similar to those seen on lettuce plants serving as positive controls. The inclination to label endive and others as hosts of *X. c*. pv. *vitians* has been tempered however, by a lack of field observations of bacterial leaf spot on these plants. Evidence of differences in titration end points leads us to conclude that lettuce is clearly different in its host/parasite relationship to *X. c.* pv. *vitians* than are the other crop species tested. It may be that resistance to bacterial leaf spot in endive and similar plants is a lower level of

resistance that is controlled by a number of genes (Parleviet and Zadoks 1977), rather than a typical gene-for-gene hypersensitive resistance (Flor 1942; Flor 1971). At inoculum concentrations of 10^7 cfu/ml or greater, this generalized resistance is overcome under greenhouse conditions (Staskawicz *et al.* 1995). Absence of symptoms in endive and other non-lettuce crops in the field may not be surprising given the inoculum threshold necessary to incite disease.

Infectivity titrations have been used to help identify cultivars of crops that have levels of resistance to a number of bacterial pathogens. For example, Chamberlain (1962) was not able to see differences in susceptibility of soybeans to *X. campestris* pv. *glycines* until inoculum concentrations were reduced to 10^5 cfu/ml. Similarly, Foster and Echandi (1973) found that high inoculum concentrations obscured resistance in *Lycopersicon hirsutum* accessions to *Clavibacter michiganensis* subsp. *michiganensis.* They suggested screening for resistance to this tomato pathogen at 10^5 CFU/ml.

We suggest that an array of tactics be used to determine the host range of *X. c.* pv. *vitians* among the diverse crops now grown in the EAA. These can include *in vivo* population dynamics studies, measurement of epiphytic populations on leaves in the field, and the infectivity titration determinations as described above.

CHAPTER 7
CONCLUSIONS

By placing lettuce plants inoculated with *X. c.* pv. *vitians* in growth chambers at 15, 20, 25, and 30º C and observing them for severity of disease over time, a mathematical relationship describing the effect of temperature on disease development was determined. A quadratic equation ($y=-5695.29 + 588.20x – 12.904 x^2$) helps describe the relationship. Mathematical manipulation of the equation showed that the optimum temperature for disease development was 22.7ºC.

The effect of wounding on disease development is inconclusive at this time. Few lesions were found on the host plant when inoculated. With limited data, it was found that wounding with sand did not increase the severity of disease in lettuce. Cabbage, tatsoi, and lettuce showed an increase in the severity of disease when left unwounded compared to wounded plants. This data is misleading, however, because Pernezny *et al.* (personal communication, 2003) reportedly achieved optimum disease development when plants were overhead irrigated. If plants had been overhead irrigated in these experiments, perhaps the amount of disease would have been higher on the host plant and would have given more conclusive results.

Internal populations of *X. c.* pv. *vitians* were highest in lettuce compared to the other inoculated plant species. Populations were near 10^8 CFU/cm^2. Pepper also supported rather high internal populations, just a log lower than lettuce, supporting the thesis of Sahin and Miller (1997) that pepper is likely an alternative host of *X. c.* pv. *vitians*. Populations on tomato, parsley, cilantro, and beet were as much as four logs

lower than those of lettuce. Therefore it is unlikely that these crops are epidemiologically important hosts of epiphytic populations of *X. c.* pv. *vitians*.

Highest epiphytic populations were found on asymptomatic leaves of lettuce and tomato in the field study. By the fifth sampling period, bacterial populations were detectable only on tomato while no bacteria were detectable on all other plant species. Bacterial populations on endive stayed high until the third sampling when they fell to levels similar to those in beet, parsley and cilantro. The environmental conditions at the time of the test were not favorable for the growth of epiphytic bacteria. For example, there was very little rain. Taking into account the inhospitable environmental conditions, this study supports the possibility of tomato serving as a reservoir of epiphytic populations of *X. c.* pv. *vitians*.

An increase in knowledge regarding the growth of *X. c.* pv. *vitians* on different crop species which may be grown near to one another due to the increase in demand for mixed salads could be pertinent to the growers of Florida. Being aware that endive, tomato, and pepper support higher populations than beet, parsley, and cilantro could decrease the chances of spread of the disease from one plant species to another if growers use a cropping regime in which these cropping combinations are avoided. Knowing that the optimal temperature for disease development is around 22.7º C and that an increase of rainfall increases the severity of BLS is important information for Florida growers. It would be useful for a grower to be vigilant when temperatures are low and moisture is high.

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