THE ROLE OF TANOS AND ITS COMPONENTS IN THE MANAGEMENT OF BACTERIAL SPOT OF TOMATO AND PEPPER, AND BACTERIAL LEAF SPOT OF LETTUCE

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To my parents, Natividad Codio and Gerard Fayette who have always provided the best for my personal and professional growth

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Tank mixes of pesticides are often used in plant disease control. The effects of Tanos and its components, Famaxadone and Cymoxanil, in combination with copper and mancozeb in some instances, were evaluated on the *in vitro* growth of strains of *Xanthomonas perforans, X. euvesicatoria, X. campestris pv vitians*, on disease control of bacterial spot of pepper and tomato in the greenhouse, and on the population dynamics of Cu^r strains of *X. perforans* and *X. euvesicatoria* on tomato leaves and pepper leaflets.

Both *in vitro* and greenhouse trials demonstrated that Tanos and its components do not have any bactericidal activity. In some instances, Tanos tends to promote bacterial growth. However, the addition of Tanos to the copper/mancozeb mixture tends to induce a modest synergistic increase in the toxicity of the combination. It appears that both components of Tanos, Cymoxanil and Famaxadone, are essential for synergistic interaction since the addition of Cymoxanil or Famaxadone to copper did not induce significant reduction of bacterial populations in comparison to copper alone. In the greenhouse trials, levels of disease control were similar for copper alone, and

copper/Tanos in most the trials. In some instances, the mixture copper/Tanos led to similar level of disease control as the standard mixture of copper/mancozeb, and copper + Tanos + Mancozeb.

Tanos and its components do not have bactericidal activity against the epiphytic populations of *X. perforans*, and *X. euvesicatoria* on tomato leaves and pepper leaflets. Different mixtures with copper, Famaxadone, Cymoxanil, and Tanos generally did not differ at stastical level. In one trial with a strain of *X. perforans,* the standard mixture copper/mancozeb did consistently reduce the pyllosphere populations in each sampling period in comparison to the control.

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CHAPTER 1
BACTERIAL SPOT OF PEPPER AND TOMATO

Vegetable production is one of the most important agricultural activities in Florida. This state ranks first in the United States in terms of fresh-market tomato and pepper value. U.S consumption of fresh tomatoes increased 71%, from 7.0 kg per capita in 1991 to 9.4 kg per capita in 2006. In 2007, the Florida tomato industry was valued at $ 464 million (USDA, ERS, 2008a). The country relies heavily on Florida for the supply of fresh peppers from October through June. In 2007, Florida harvested 17 500 acres of bell pepper, valued at over $ 183 million (USDA, ERS, 2008b).

Both crops are affected by many diseases. Bacterial spot is one of the major ones and occurs wherever tomato and pepper are grown. It is particularly troublesome in tropical and subtropical areas (Jones et al., 1998a). Production is affected by the disease, which can result in great economic losses. In some fields, loss of foliage may fluctuate between 50-70% (Pohronezny and Volin, 1983). Crop losses result from the reduction in yield due to defoliation and severely spotted fruits, which are not suitable for the market (Jones, 1991). Bacterial spot is a major concern to transplant growers because many states that receive seedlings from Florida require these transplants to be free of the pathogen. (Sun et al.,2002).

History and Strain Diversity

Bacterial spot disease of tomato (*Solanum lycopersicum*) was first observed in 1914 in South Africa and described as a tomato canker by Ethel Doidge (1920). The causal agent was named *Bacterium vesicatorium*. Gardner and Kendrick (1921) described a similar disease in the United States and referred to it as bacterial spot. They named the causal bacterium *B*. *exitiosum*; Doidge's name took precedence . A

similar disease on pepper (*Capsicum annum*) was described by Gardner and Kendrick (1923). Numerous studies showed that the bacterial pathogens from pepper and tomato used in the initial studies induce disease in both plants, and it was believed for many years that cross infection could occur in field (Stall et al.,, 2009).

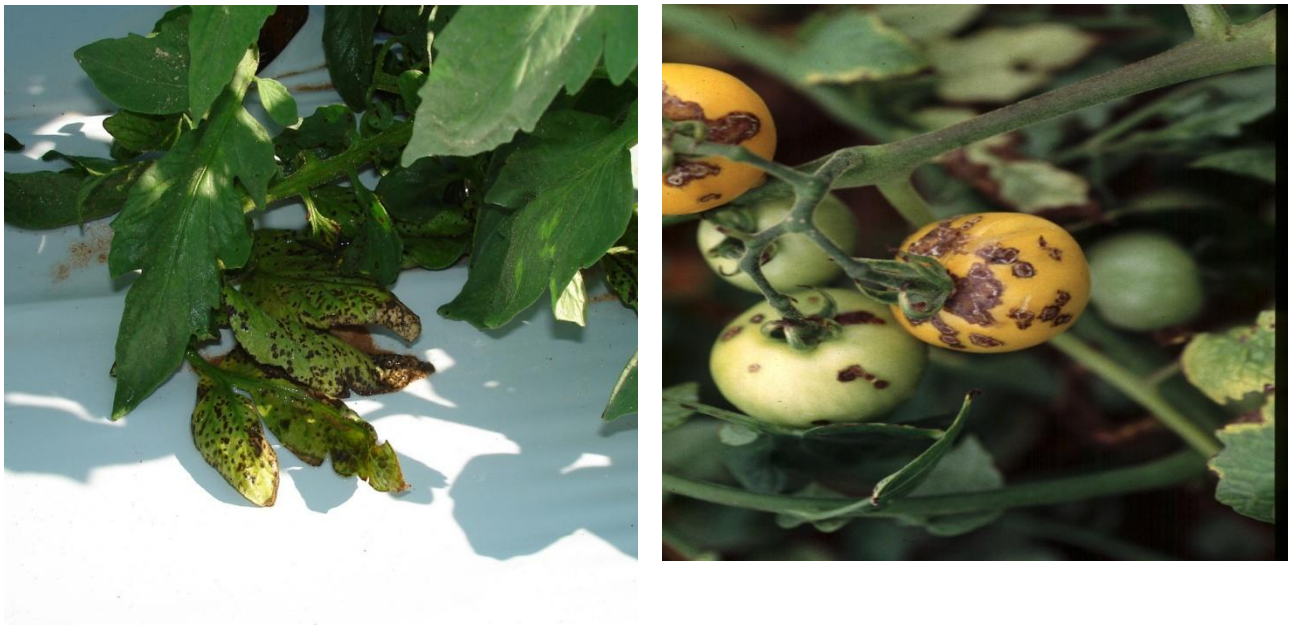


Figure 1-1. Symptoms of bacterial spot on pepper leaves and tomato fruits

Symptoms induced by these different xanthomonads are similar. The characteristic symptoms include the development of small, brown to black lesions of 1-3 mm diameter, with or without yellow halos, that affect all the aboveground organs (Figure 1-1). Subsequent enlargement and coalescence of spots occur later, which leads to the browning of the entire leaf and defoliation (Kucharek, 1994). However, there are some small differences, such as a shot-hole symptom induced by *X*. *perforans* on tomato (Stall et al.,, 2009). The pathogen can be readily isolated from infected tissue. It is a gram-negative, rod-shaped bacterium. It is motile with a single polar flagellum, strictly aerobic, and measures 0.7 to 1.0 µm by 2.0-2.4 µm. Other

characteristics include production of a yellow, water-insoluble pigment, xanthomonadin and an extracellular polysaccharide (EPS) named xanthan.

Based on pathogenicity profiles, many races have been identified. A race is characterized by its ability to grow on a cultivar with or without specific genes for resistance (Pernezny et al. 2008a). The letters P, T and PT are respectively assigned to races pathogenic to pepper only, tomato only or both pepper and tomato (Gore and O'Garro, 1999). All *Capsicum* genotypes tested are resistant to strains of *Xanthomonas* that are only pathogenic to tomato, and conversely, all *Lycopersicon* genotypes evaluated show resistance to strains of the pepper group. Thus, specific genotypes of pepper and tomato have been used to characterize races of xanthomonads pathogenic to theses hosts (Table 1-1). Both genera (*Capsicum* and *Lycopersicon*) contain genes for resistance to *Xanthomonas*, but the genes cannot be transferred between these genera through natural hybrizidation (Jones et al., 1998). Several avirulence genes have been identified in xanthomonads associated with tomato. Strains of *X. euvesicatoria,* tomato race 1 (T1), carry the avirulence gene *avrRxv* , which induces a hypersensitive response (HR) on the genotype genotype H7998 with the corresponding resistance gene *Rxv* (Whalen et al. 1993). Strains of *X. perforans*, tomato race 3 (T3) contain the *avrXv3* that induce an HR on H7981 (Minsavage et al., 1996). Another avirulence gene, *avrXv4,* was found *in X. perforans* strains based on HR reactions with the *Xv4* resistance gene of the tomato genotype LA716 (*Lycopersicum pinnellii*). Thus, strains of *X. perforans*, containing the *avrXv4,* but lacking a functional *avrXv3,* were designated tomato race 4 (Astua-Monge et al., 2000a; 2000b).

Table 1-1. Resistance in tomato and pepper and avirulence genes that interact with them

	Resistance		Bacterium	
	Source	Species	Effector	Location
Pepper				
Bs1	PI 163192	*C.a annuum*	AvrBs1	Plasmid
Bs2	PI 260435	*C. chacoense*	AvrBs2	Chromosome
Bs3	PI 271322	*C. annuum*	AvrBs3	Plasmid
Bs4	PI 235047	*C. pubescens*	AvrBs4	Plasmid
Bs5	PI 163192 or	*C. annuum*	NDc	ND
	PI 271322	*C. annuum*	ND	ND
Bs6	PI 163192	*C. annuum*	ND	ND
	PI 271322	*C. annuum*	ND	ND
BsT	Commercial pepper	*C. annuum*	AvrBst	Plasmid
Tomato				
rx1,rx2,rx3	Hawai 7988	*S.b lycopersicum*	AvrRxv	Chromosome
Xv3	PI128216 & Hawai 7981	*S. pimpinellifolium & S. lycopersicum*	AvrXv3	Chromosome
Xv4	716	*S. pennellii*	AvrXv4	Chromosome
Bs4	Commercial tomato	*S. lycopersicum*	AvrBs4	Plasmid

Source : Stall et al., 2009

[a] Capsicum

[b] Solanum

[c] not determined

Several avirulence genes, with their corresponding resistance genes, have also been characterized in pepper (Table 1-1). Ten races of *X. euvesicatoria* have been identified based on hypersensitivity reactions with the resistance genes *Bs*1*, Bs*2*, Bs*3 and *Bs*4 (Stall et al.,,2009).

Tomato race 1 (T1) was commonly found in Florida before the appearance of T3 in Florida in 1991. In vitro tests showed that T3 strains are antagonistic to T1 strains (Jones et al., 1997). Tudor (1999) determined that T3 strains produce bacteriocin-like substances that have biological activity against T1 strains. The antagonism of T3 against T1 may lead to a shift in race composition towards T3 strains in Florida, with a

bacteriocin production confering a competitive advantage for T3 strains (Jones et al., 1997).

In 1982, pepper race 2 (P2) was the primary race in Florida (Cook and Stall, 1982), but by the late 1980s, pepper race 1 (P1) had become the prevalent race in Florida. In 1988, 89% of the strains were represented by P2, but in 1989, P2 strains accounted for only 15% (Pohronezny et al., 1992).

Shifts in race populations can occur several ways: introduction of a new race by seeds or other plant propagative materials, introduction of resistant cultivars which may select for new races, and competitive advantage (Jones et al., 1997). Based on the widespread use of copper-based bactericides in Florida, it appears that copper resistance gave P2 strains a competitive advantage (Jones et al., 1997), which could explain why P2 strains were prevalent before 1989 in Florida (Pohronezny et al., 1992).

It has been proposed that specific races were associated with specific regions (Cook and Stall, 1982). However, other reports mention the establishment of the same races in a number of locations (Jones et al. 1995; O'Garro and Tudor, 1994). It has been suggested that the introduction of pepper and tomato seeds may serve as sources of races that eventually became endemic in production (Pohronezny et al.,1992). The pathogen race structure may also be altered by mutation and selection. A report referred to the emergence of the race P3 from P1 and P2 by plasmid loss (Kousik et al., 1996).

Epidemiology

A number of different sources of primary inocula have been identified for the bacterial spot pathogen. The spectrum of hosts of bacterial spot is not limited to peppers and tomatoes, but also includes a few other solanaceous plants (Stall et al.,

2009). The pathogen may stay alive between seasons in lesions on volunteer plants and it does not remain in the soil without crop debris for more than 6 weeks in the Florida summer. Seeds may be an important source of inoculum (Jones, 1991). Commercial seeds are usually treated with a bactericide in order to reduce seedborne primary inoculum (Stall et al., 2009). A low incidence of contaminated seed can induce a high incidence of disease in the field due to epiphytic multiplication and distribution of the pathogen in the phyllosphere (Louws et al. 2001). Cotyledon leaves generally become infected after the emergence from an infested seedcoat. Seedlings and transplants become infected by rain splash or windblown particles from nearby infected plants. Infected transplants carry the bacterial inocula to the field and there may induce further contamination (Jones, 1991).

The bacteria can enter the plant when there are favorable conditions for disease development, including a threshold epiphytic population or significant wounding wounds engendered by wind-driven sand, insect punctures or mechanical means facilitate the ingress of the pathogen (McGuire et al. 1991; Jones, 1991). Natural opening such as stomates and hydathodes can also serve as an entry point (Ramos and Volin, 1987). High humidity is also an important factor; high humidity has been shown to increase infection of *X. euvesicatoria* by 10- to 100- fold on tomato leaves in comparison to low humidity (Timmer et al.1987).

Disease Control

Cultural practices such as good field sanitation (Pohronezny et al. 1990), crop rotation, the use of disease- and pathogen-free transplants and the elimination of solanaceaous weeds such as ground cherry and nightshade reduce primary inoculum(Kucharek, 1994). Cultural practices alone are not sufficient for good control

and need to be complemented with other approaches. Streptomycin was commonly used in the 1950s for the control of bacterial spot, but was no longer recommended by the 1960s due to resistance by the bacteria to this antibiotic (Stall and Thayer, 1962). Other approaches to management include the application of bacteriophages mixes (Flaherty et al. 2000; Balogh et al. 2003) , activators of the plant immune response that induce systemic- acquired resistance such as acibenzolar-S-methyl (Louws et al. 2001). For many years, the standard recommendation has been the application of copper compounds. In various studies, it has been shown that the addition of maneb or mancozeb to copper bactericides increases their bactericidal activity. It is important to note that the carbamates, mancozeb or maneb did not control the bacteria when used alone (Marco and Stall, 1983). In some instances, copper-mancozeb mixtures resulted in a reduction of bacterial populations on tomato leaves and improved disease control (Jones et al., 1991). However, this combination was not effective when weather conditions were optimal for disease development; positive yield responses were rarely obtained in conditions where copper-resistant strains were present (Jones and Jones, 1985).

Copper-Resistant Strains

Foliar application of chemicals such as fixed copper compounds has been routinely used to try to control the disease. Different copper compounds: cupric hydroxide, tribasic Cu sulfate, Cu ammonium carbonate, Cu oxychloride sulfate, and Cu salts of fatty and rosin acids; are used in the control of bacterial spot . The use of these chemicals has led to the frequent occurrence of copper-resistant strains of vegetable bacterial pathogens (Marco and Stall, 1983; Bender et al., 1990; Cooksey, 1990).

Two apparently independent lines of copper resistance genes in xanthomonads associated with bacterial spot pepper and tomato are known. Copper-resistance genes were found on 188- to 200-kb self-transmissible plasmids in strains from Florida and Oklahoma (Bender et al., 1990; Stall et al.,, 1986) and on a 100-kb non-self transmissible plasmid in a strain from California (Cooksey et al.,1990). Chromosome-encoded copper resistance was found in a strain XvP26 from Taiwan which contains a small plasmid (15 kb) (Basim et al. 1999; Canteros et al.,1995).

Bactericides have been used in plant disease control for decades. Nevertheless, in the 1980s, resistance to the most commonly used bactericide, copper, was detected (Marco and Stall, 1983). It may be that copper resistance occurred much earlier but was overlooked by plant pathologists (Cooksey,1990). Moreover, the widespread combination of an ethylene bisdithiocarbamate fungicide with copper, which enhances the toxicity of copper sprays, might not lead to earlier detection of copper-resistant pathogens in the field (Marco and Stall, 1983). It seems that resistance has been present in the field for many years since strains isolated in 1968 were found to be copper-resistant (Cooksey, 1990).

The increasing use of antimicrobial agents has led to a strong selective force, favoring the survival of bacterial strains to such agents, either by mutation or by acquisition of R-plasmids (Davies and Smith, 1978). In laboratory assays, chromosomal mutations for bacterial resistance can be induced, but the role of such resistance in field populations is not well understood (Cooksey, 1990). In natural isolates of most bacteria, metal-and antibiotic-resistance genes are usually found on plasmids and transposons (Cooksey, 1994; Silver and Misra, 1988). Canteros et al., (1995) determined the

plasmid profile of 522 strains of xanthomonads associated with bacterial spot of pepper and tomato. They were from both culture collections from different geographic locations and strains isolated from commercial fields in Florida. High diversity, in terms of number of plasmids and plasmid size (3 to 300 kb), was observed. Such diversity could be the result of frequent plasmid transfer between bacterial strains within the pyllosphere.

It is important to note that plasmid-borne resistance determinants are easier to identify through conjugal plasmid transfer to bactericide-sensitive strains (Cooksey, 1990). Both mechanisms of copper-resistance gene evolution may be correlated in some species. For example, chromosomal genes were found to be similar to plasmid-borne copper-resistance genes in pseudomonads, and such genes are involved in copper uptake and management in *Escherichia coli* (Cooksey, 1993).

The presence of these plasmids possibly provides a selective metabolic advantage to the bacterial strains in comparison to their plasmid-free relatives and also provides extra genetic material. This material may be involved in antibacterial resistance that cannot be linked to the mutation of host chromosomal genes (Davies and Smith, 1978).

Evaluation of Copper-Resistant Strains

The existence of copper-resistant strains in the fields can be inferred from the poor control of bacterial spot with applications of copper bactericides at recommended rates (Martin et al., 2004). The continuous application of such pesticides results in selection of resistant strains until a resistant population becomes an important component in disease epidemics (Martin et al., 2004). However, in the absence of selection pressure, copper-resistant strains can revert to copper sensitive ones (Gore and O'Garro, 1999).

The basis of the reversion might be due to the loss of plasmids encoding copper resistance (Stall et al.,,1986).

Several methods have been reported in the literature to assess the resistance among bacterial strains (Gore and O'Garro, 1999; Marco and Stall, 1983; Zevenhuizen et al. 1979; Pernezny et al. 2008). Stall et al., (1986) found plates of nutrient agar amended with 200 µg/ml $CuSO_4.5H_20$ were useful in the screening of resistance to copper among pepper strains of *Xanthomonas euvesicatoria*. Strains from Barbados, associated with bacterial spot of pepper and tomato, that produced confluent growth on nutrient agar amended with 200 µg/ml $CuSO_4.5H_20$, were considered resistant to copper and those that failed to grow were considered sensitive (Gore and O'Garro, 1999). This procedure (Stall et al., 1986) was used to assess the copper resistance of tomato strains of *Xanthomonas perforans* (Jones et al. 1991) and strains of *Xanthomonas campestris pv. vitians* (Pernezny et al. 1995). In Australia, Martin et al. (2004) used a low-complexing casitone yeast-extract-glycerol broth medium to determine copper tolerance. This medium is characterized by minimal tendency to bind the copper to components of the medium. The rationale for this choice is the fact that most of the copper remains in the ionic form, thus ensuring maximum toxicity to the bacteria. Copper-resistant bacterial strains survived at 1.0 mM $CuSO_4$ in this medium. (Zevenhuizen et al. 1979). Marco and Stall (1983) reported sensitivity to copper based on the viability of cells after exposure to copper solutions. Sensitive strains were killed in suspensions in which the concentrations of soluble amount of copper were 1-2 mg/L. A concentration of 13 mg/L was necessary to kill the copper-resistant strains.

Pernezny et al. (2008b) reported that the protocol and especially the culture medium chosen to screen bacterial strains can affect the classification of strains based on sensitivity to copper. In their study, these authors found that most of the strains were classified as resistant when using glucose nutrient agar (GNA) amended with copper (Stall et al., 1986) and sensitive when using casitone-yeast extract, CYE, (Andersen et al. 1991) amended with copper. Their results support GNA + Cu as a more suitable medium to screen *Xanthomonas* strains from Florida. However, another factor that may influence the choice of the medium for screening for copper resistance is the host/pathogen system (Pernezny et al. 2008b). CYE amended with copper was useful to classify *Pseudomonas cichorii* strains into highly resistant, moderately resistant and sensitive strains (Pohronezny et al. 1994).

CHAPTER 2
BACTERIAL LEAF SPOT OF LETTUCE

Bacterial leaf spot of lettuce, *Lactuca sativa* L, (BLSL) was first reported in 1918 in the United States (Brown, 1918). She proposed that the causal agent be named *Bacterium vitians*. Since the first report, the disease has been observed in many states including Florida (Pernezny et al.,1995), Ohio (Salin and Miller, 1997), and California (Barak et al., 2001). They also have been reports from India (Wallis and Joubert, 1972), France (Allex and Rat, 1990), Canada (Toussaint, 1999) and Turkey (Sahin, 2000).

In 1951, Elliot reported that three xanthomonds are associated with BLSL: *Xanthomonas vitians, X. lactucae* and *X. lactucae-scariolae* (Elliot, 1951). Further characterization showed that these three described species were not distinct, but rather synonyms for *X. vitians* (Burkholder, 1954). In 1995, a reclassification of xanthomonads was proposed that include 20 *Xanthomonas* DNA homology groups (Vauterin et al., 1995). Based on DNA-DNA hybrizations and Biolog profiles, the current taxon *Xanthomonas campestris* pv *vitians* was divided in two groups, strains of A and B. (Vauterin et al. 1995). Using tetrazolium violet as a redox indicator, the Biolog system identifies bacteria based on metabolic activity in the presence of 95 different carbon sources (Toussaint, 1999). Group B strains, including the pathovar reference strain LMG 938, renamed *X. hortorum* pv vitians, show high relatedness with *X. campestris* pv*. pelargonii* and *X. campestris* pv*. hederae*. The pathovar reference strain LMG 937 was the only strain that was included in the taxon *X. axonopodis* pv*. vitians* and fell into Group 9 strains that include 34 *X. campestris* pathovars and *X. axonopodis* (Vauterin et al. 1995). However, Stefani et al. (1994) and Barak et al. (2001) reported that the strain LMG 937 was not pathogenic on lettuce. Sahin et al. (2003) also found that the strain

LMG 937 was nonpathogenic on lettuce, but was weakly pathogenic on tomato and pepper, inducing small numbers of necrotic spots. This strain, isolated in 1917, might be misidentified, mislabeled or might have lost pathogenicity on lettuce after many years in storage (Sahin et al. 2003).

Table 2-1. Difference among *Xanthomonas campestris pv vitians* and *Xanthomonas* sp. strains, based on carbon sources in Biolog GN microplate assay (Sahin et al. 2003)

	X. campestris pv. *vitians*		*Xanthomonas* sp.
Carbon substrate	Group A	Group B	LMG 937
α -D-Lactose	-	-	+
D-Melibiose	+	+	-
D-Raffinose	+	+	-
Formic acid	-	-	+
α -Hydroxybutyric acid	-	+	+
α -Ketobutyric acid	-	+	+
Glycyl-L-aspartic acid	-	-	+
L-serine	+	-	+
L-Threonine	-	-	+
Glycerol	-	-	+

Despite the uncertain affinity of the reference strain LMG 937, a recent study supports the separation of *X. campestris* pv*. vitians* strains into at least two groups (Table 2-1) (Sahin et al. 2003). Group A strains cause both local and systemic symptoms, whereas Group B strains, including the pathovar reference strain LMG 938, induced only distinct necrotic spots. Due to their systemic spread in the plant, Group A strains may represent a greater threat to lettuce production. It was also found that the *X. campestris* pv*. vitians* type strain, LMG 937, and California strain B-53, both isolated from lettuce, are different from Group A and B strains and were not pathogenic on lettuce. Such separation was supported by monoclonal antibodies, fatty acid methyl ester analysis (FAME), sodium dodecyl sulfate polyacrylamide gel electrophoresis

(SDS-PAGE), repetitive extragenic palindromic (Rep-PCR) fingerprinting studies. However, there were no differences in the sequences of 16S-23S rDNA spacer regions of four representative strains (Sahin et al. 2003). Barak et al. (2001) also found that the spacer regions of strains from different geographical origins were identical, but no genetic evidence of different groups pathogenic on lettuce. Analysis of ribosomal RNA is a well established tool to study the relationship among bacteria (Woese, 1987). Due to the high level of sequence conservation that can exist among rRNA genes (16s, 23S and 5S) at the genus and species levels, the spacer regions-variable sequences separating these genes can be a useful taxonomical tool (Jensen et al. 1993). Several pathovars of *Pseudomonas syringae* could not be differentiated on the basis of RFLP analysis of PCR-amplified *rrs* (16S) and *rrl* (23S) genes. However, *P. syringae* pv. *tomato* strains were differentiated from other pathovars based on RFLP analysis of the internal transcribed spacer region 1 (ITS1) (Manceau and Horvais, 1997).

The nomenclatural change proposed by Vauterin et al. (1995) has not be fully accepted by the scientific community. Schaad et al. (2000) rejected the reclassification of *X*. *campestris* pv. *vitians* type A as *X*. *axonopodis* pv. *vitians* and *X. campestris* pv. *vitians* type B as *X. hortorum* pv. *vitians* until more phylogenetic information is available. Recent literature continues to identify the pathogen as *X. campestris* pv. *vitians* (Pernezny et al., 2002; Robinson et al., 2006). In this thesis, the causal agent of Bacterial leaf spot of lettuce (BLSL) is referred as *X. campestris* pv. *vitians* (Xcvi).

Economic Impacts

Bacterial leaf spot reduces the quality and yield of lettuce and increase the risks of postharvest losses (Carisse et al. 2000). Bacterial leaf spot have been reported in fields of all major market types of lettuce including leaf, crisphead, butterhead and romaine

(Pernezny et al., 1995; Toussaint, 1999; Carisse et al., 2000). In Florida, BLSL continue to be a major concern for farmers because of the favorable conditions for disease development in the subtropical climate of southern Florida (Pernezny et al. 2002). Under favorable conditions, the disease can damage head leaves of crisphead lettuce, making the produce unmarketable (Toussaint, 1999).

Symptoms

The pathogen induces small, angular leaf lesions, about 1-2 mm in diameter, along the margin of leaves, which are water-soaked, dark brown or olive colored. The lesions become V-shaped, translucent and progress along the veins (Sahin and Miller, 1997). Coalescence of lesions results in large necrotic regions (Bull et al. 2007). Another type of symptom consists of individual black spots dispersed on the leaf surface (Sahin and Miller, 1997). Plants with BLSL symptoms are more susceptible to other fungal diseases including *Botrytis cinerea, Sclerotinia sclerotiorum* and *Rhizoctonia solani* (Carisse et al. 2000; Toussaint, 1999).

Epidemiology

Warm, humid, and rainy environmental conditions are conducive for the development of BLSL (Barak et al. 2001; Toussaint, 1999). There is some inconsistency related to the reports of optimum temperature for infection. Brown (1918) found the optimum temperature for *in vitro* growth of Xcvi ranges from 26 to 28 ºC. Toussaint (1999) reports that the optimal growth temperature *in vitro* is 28 ºC. Based on growth chamber studies, Robinson et al. (2006) determined that the optimum temperature for infection was 22.7 ºC. Robinson et al. (2006) suggested to collect strains from different areas in order to compare temperature optima, since the differences observed in the reports may be related to variation among strains from different locations.

X. *campestris* pv *vitians (Xcvi)* can have an epiphytic stage on leaf surfaces before the induction of symtoms, based on scanning electron microscopy of asymptomatic leaves infected by *Xcvi* strains or by dilution plating after the maceration of asymptomatic leaves in phosphate buffer (Toussaint, 1999). Sahin and Miller (1997) found relatively high populations of *Xcvi,* ranging from 10^9 to 10^{12} CFU/ g fresh weight leaf tissue, on leaves inoculated with a concentration of 10^8 CFU/mL. They concluded that some of the detected populations result from bacteria surviving epiphytically on leaf surfaces.

The pathogen is also known to survive on diseased plant debris for short periods of time and in association with weeds (Barak et al. 2001; Sahin and Miller, 1997). Barak et al. (2001) found that populations of *Xcvi* can colonize and survive in association with crop debris for at least 5 months in California. Contaminated debris can serve as an inoculum source for subsequent crops. Contaminated seeds are also an important source of inoculum and are also the major mean of long-distance dissemination of the bacterium (Sahin and Miller, 1997). However, the bacteria have not been consistently recovered from commercial seed lots. Contaminated debris may be more important for BSLS development than seedborne inoculum (Barak et al. 2001). Jones et al. (1986) reported a similar situation for bacterial spot of tomato, in which inoculum sources other than seed, such as volunteer tomato plants and crop residue, appear to play a more important function in the epidemiology of the tomato disease.

Control

For an effective management of BLSL, an integrated approach is needed. Management tactics should include seed treatment, crop rotation, elimination of wild host plants in or around lettuce fields, and avoidance of overhead irrigation. Other tools

are copper-based fungicides in association with mancozeb and the use of disease-resistant cultivars (Sahin, 1997; Toussaint, 1999; Carisse et al. 2000).

Pernezny et al. (1995) reported that the romaine (cos) and butterhead types were more susceptible to BLSL than crisphead lettuce. Sahin and Miller (1997) reported that only the red leaf cv. Redline, among nine commercial cultivars grown in Ohio, was resistant to the disease. Carisse et al (2000) also found that butterhead and cos types were the most susceptible cultivars, with the green-leaf types less susceptible.

Since seeds are probably a major source of inoculum, seed treatment is an essential component of an integrated management program. Several seed treatments are suggested. Carisse et al. (2000) found that the most efficient seed treatment was a 1% of sodium hypochlorite soak for 5 to 20 min. Pernezny et al. (2002) reported that seedborne inoculum was reduced below 10% with a 1% sodium hypochlorite treatment, but they recommended a 15 minutes soaking time for better efficacy. They also also found that a mixture of copper hydroxide and mancozeb and solutions of aqueous 3 to 5% hydrogen peroxide effectively eradicated *X*. *campestris* pv *vitians* associated with lettuce seeds.

CHAPTER 3
CHEMICAL CONTROL AND INTERACTIONS BETWEEN FUNGICIDES

There are few bactericides among the increasing numbers of manufactured pesticides (Mew and Natural, 1993). Chemical control of many bacterial diseases has been in general a major challenge. Pathogen variability, high risk for development of resistant strains, rapid population growth, and few available chemical-based options all contribute to the difficulty in management of bacterial diseases (Jones et al. 2007).

The discovery of Bordeaux mixture in 1880's is considered to be an important step in the history of chemical control. Bordeaux mixture is part of the first generation of fungicides, which also includes other inorganic chemicals. The development of dithiocarbamates constitutes the second generation of fungicides (De Waard et al., 1993). These fungicides are surface protectants. They do not enter the plant tissues and are only effective if applied in advance of infection (Sbragia, 1975). Third-generation fungicides (e.g.: benzimidazoles, carboxamides, phenylamides) are mainly systemic and help in the control of established infections (Waard et al., 1993; Baldwin and Rathmell, 1988). The fourth generation of fungicides (e.g.: Trycycazole, probenazole) includes compounds that are non toxic in in vitro trials, but control plant disease by interfering with processes involved in pathogen penetration, or by enhancing plant defense responses (Waard et al., 1993).

Antibiotics, such as streptomycin, have been used in agriculture to control phytopathogenic bacteria (Mew and Natural, 1993). Extensive use of this antibiotic has increased the prevalence of streptomycin-resistant strains in bacterial populations, which reduces the efficacy of streptomycin-based control (Cooksey, 1990), including bacterial spot of tomato and pepper (Thayer and Stall, 1961). However, the agricultural

use of antibiotics with medical applications is discouraged due to potential transfer of resistance genes from phytopathogenic bacteria to those associated with animals and humans (Mew and Natural, 1993).

Interactions Between Fungicides

Tank mixes of pesticides are often used in plant disease control. Pesticides are combined in order to widen the spectrum of biological activity, to delay the selection of resistant strains, and exploit synergistic interactions (Gisi et al., 1985). The efficacy of a mixture may be equal to the additive effects of the single substances, or may be sometimes superior or inferior to these additive effects. (Scardavi, 1965; Samoucha and Cohen, 1986). Synergism is referred as a phenomenon in which the total response of an organism to the mixture is higher than the sum of responses to the individual components (Scardavi, 1965). When the efficacy of the mixture is below the arithmetical sum of the effects of individual components, it is referred to antagonism (Samoucha and Cohen, 1986).

Several synergistic interactions between fungicides have been reported in the literature (Gisi et al. 1985; Marco and Stall, 1983; Roberts et al. 2008). Synergistic interactions occured when oxadixyl, mancozeb and Cymoxanil, were mixed in different concentrations against sensitive strains of *Phytophthora infestans* in vivo (Gisi et al. 1985). Marco and Stall (1983) reported that the mixture of copper and mancozeb induced a better control of bacterial spot of pepper than copper alone. The mechanism by which this enhancement of copper toxicity occurs is unknown; however, one suggestion is that the EBDC fungicide may induce an increase in the amount of soluble copper (Cooksey, 1990). In vitro tests showed that mancozeb increased the soluble copper in the suspension (Marco and Stall (1983). Roberts et al. (2008) reported

synergistic interactions between Tanos and copper, including mancozeb in some instances, in the control of bacterial spot of tomato.

Mode of Action of Copper, Tanos, Cymoxanil and Famaxadone

Copper

Copper is an integral part of many enzymes involved in many vital processes. Copper serves as a protein cofactor in fundamental redox reactions that involve enzymes such as cytochrome oxidase and superoxide dismutase (SOD). It is vital in cellular respiration and free radical defense mechanisms (Harris and Gitlin, 1996). Normally bound to proteins, Cu may be released and become free and serves as a catalyst in the formation of highly reactive hydroxyls radicals (Gaetke and Chow, 2003). Thus, if levels of free ions increase, a number of toxic effects can occur in cells (Cooksey, 1994). Copper is generally biocidal, affecting plants, fungi and bacteria. Cupric ion, Cu^{2+}, is the toxic form. It denatures proteins and competes with essential metals for biding sites on coenzymes. Copper, at concentrations higher than 1 µM, is a potent inhibitor of photosynthetic electron transport (Mohanty et al., 1989).

Mancozeb

The organic sulfur compounds comprise one of the most important and versatile group of fungicides. They include thiram, ferbam, nabam, maneb, zineb and mancozeb. These fungicides derive from dithiocarbamic acid. It is believed that the dithiocarbamates are fungitoxic because they are metabolized to the isothiocyanate radical, -N=C=S. This radical induces the inactivation of the sulfhydryl group (-SH) in amino acids and in enzymes within pathogen cells and consequently inhibits the production and function of these compounds (Agrios, 1997).

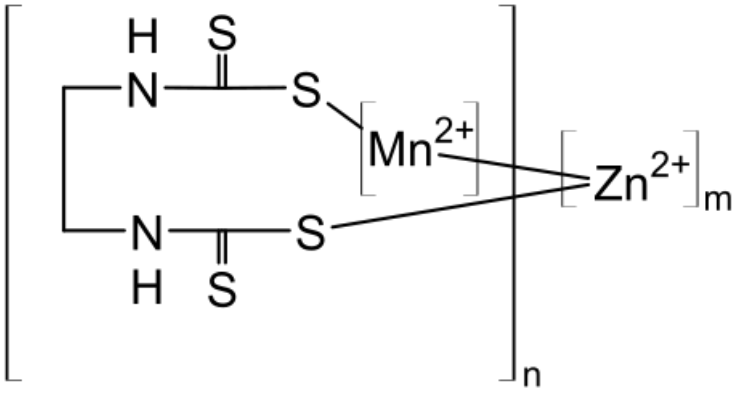
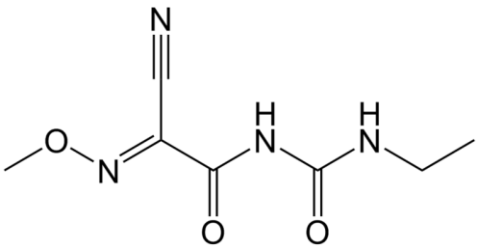


Figure 3-1. Structure of the fungicide Mancozeb

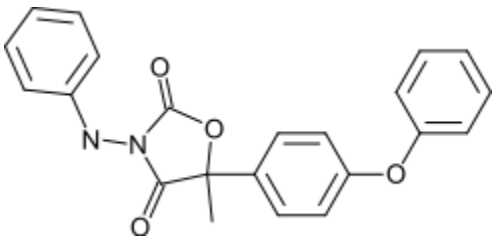
Mancozeb belongs to the ethylenebisdithiocarbamate group, which also include maneb and zineb. It is a broad-spectrum fungicide that is useful in the control of many foliage and fruit diseases of many vegetables. Mixture of maneb with zinc or zinc ion results in the formulations known as maneb zinc (sold as Manzate D). Mancozeb (sold as Manzate 200, Dithane M-45, Pencozeb) is a polymer of maneb and a zinc salt. Zineb is sold as Dithane Z-79 (Agrios, 1997).

Tanos, Cymoxanil and Famaxadone

In the U.S, Famaxadone is used in combination with Cymoxanil in the formulation of Tanos DF (water dispersible granules with 25% Famaxadone/25% Cymoxanil) for the control of various fungal diseases on fruiting vegetables, potatoes, cucurbits, and head lettuce. Famaxadone is in FRAC group 11 fungicide and belongs to the oxazolidinedione class of chemicals. It is highly inhibitory to spore germination and mycelial growth of sensitive isolates. It inhibits the fungal mitochondrial respiratory chain at Complex III, which induces a reduction in production of ATP by the fungal cells (Environmental Protection Agency, EPA, 2003).



a) Cymoxanil



b) Famaxadone

Figure 3-2. Structure of the active ingredients in the formulation of Tanos

Cymoxanil is in FRAC group 27 fungicide and belongs to the cyanoacetamide-oxime class of chemicals. The trade name of Cymoxanil is Curzate 60 DF® (E.I. du Pont de Nemours and Company, Wilmington, DE). Curzate 60 DF is to be tank-mixed with a protectant fungicide such as mancozeb (EPA, 1998). It is primarily active against oomycetes (e.g. *Phytophthora, Pseudoperonospora*). Cymoxanil induces local systemic activity and post-infection activity for the first half of the incubation period (Sujkowski et al., 1995).

CHAPTER 4
THE ROLE OF TANOS AND ITS COMPONENTS IN THE CONTROL OF BACTERIAL SPOT OF TOMATO AND PEPPER, AND BACTERIAL LEAF SPOT OF LETTUCE.

The mixture of copper and mancozeb has been used for many years for bacterial disease control in vegetables. However, there is some health concerns related to the use of these fungicides. Mancozeb (Dithane 50 DF®) is registered as a general fungicide by the U.S Environmental Protection Agency (EPA). It is a complex of manganese ethylene bisdithiocarbamate (EBDC) and zinc salt. In the presence of moisture, oxygen and biological systems, the EBDC fungicides may break down (U.S. Environmental Protection Agency, 1992). Under such conditions, they can be easily degraded with the formation of products like ethylenethiourea (imidazolidine-2-thione, ETU) (Lentza-Rizos 1990). ETU has induced cancer in animals and has been identified as a group B2 probable human carcinogen by the EPA (U.S. Environmental Protection Agency, 1992). Based on this fact, they might be some restrictions in the use of mancozeb in the future. Therefore, it is important to investigate alternative compounds for the control of bacterial spot.

A report on Tanos (Tanos 50 DF®, 25 % a.i each of Famaxadone and Cymoxanil, E.I du Pont de Nemours and Company, Wilmington, DE), mixed with copper and mancozeb in somes instances, induced equal or better control of bacterial spot in field trials than copper and mancozeb alone (Roberts et al., 2008). No significant reduction in bacterial populations was observed in laboratory assays with Tanos (Pernezny et al., 2008b). Suppression of bacterial spot is included on the label for Tanos when this fungicide is tank-mixed with a full dose of copper-based fungicides.

The objectives of this study are to:

Evaluate the effect of the combination of Tanos and Kocide on resistant tomato and pepper copper strains and a lettuce strain.

Evaluate bactericidal activity of Tanos, Cymoxanil and Famaxadone alone and in combination with Kocide.

Compare control by the mixture of Tanos and Kocide with the mixture of mancozeb and Kocide.

Materials and Methods

Strains

Strains of *X. euvesicatoria* (P6), *X. perforans* (T4) and of *X. campestris pv. vitians* (L7) were studied *in vitro* and in the greenhouse using combinations of Tanos, Cymoxanil, Famoxidone, Mancozeb with Kocide. The product Tanos and its components were also tested alone. Copper-tolerant tomato race 4 of *X. perforans* and copper-tolerant pepper race 6 of *X. euvesicatoria* were provided by J.B. Jones, University of Florida, Gainesville. *X. campestris* pv*. vitians* strain L7, isolated in 1995 from Everglades Agricultural Area, was provided by K.L. Pernezny, University of Florida. For long term storage, bacterial cultures were stored in sterile 15% aqueous glycerol solution at -70 °C. Working cultures were maintained on glucose-nutrient agar slants (GNA).

In *vitro* Assays

Inoculum and assay procedure: Bacterial suspensions were prepared using 24-h cultures in a sterile phosphate-buffered saline (Leben et al. 1968). The suspensions were adjusted to A_{600} = 0.3, which approximately equals $5x10^8$ CFU/ml. Chemical suspensions (Table 4-1) were prepared in the laboratory in sterile distilled water at dosages equivalent to those recommended for field use. With a ratio of 1:1, the bacterial

suspension was mixed with the chemical suspensions in beakers (Table 4-1). Beakers were maintained at room temperature. Every 15-20 minutes, the beakers were manually shaken during a 4-hour period. Then, suspensions were filtered with sterile Whatman no. 1 filter paper. A set of 10-fold dilutions were carried out. A volume of 50 uL from dilutions was pipetted per GNA plate and a sterile bent glass rod was used to distribute the inoculum over the plate. Plates were incubated at 28°C for 72 h and only plates with less than 400 hundred colonies were counted. There were three replicates of each treatment for each experiment. Data were logarithmically transformed (base 10) and then analyzed with the Statistical Analysis System (SAS version 9.1, SAS Institute, Cary, NC). An analysis of variance and a mean separation using the Least Significant difference (LSD) were carried out.

Table 4-1. List of the treatments and their respective rate

	Treatments	Chemical g/L
T1	Control (water)	
T2	Kocide 3000 + Manzate 75DF	1.2 + 2.4
T3	Kocide 3000 + Tanos 50DF + Manzate 75DF	1.2 + 0.6 + 2.4
T4	Kocide 3000	1.2
T5	Kocide 3000 + Tanos 50DF	1.2 + 0.6
T6	Tanos 50 DF	0.6
T7	Kocide 3000 + Cymoxanil	1.2 + 0.21
T8	Kocide 3000 + Famaxadone	1.2 + 0.46
T9	Cymoxanil	0.21
T10	Famaxadone	0.46

Greenhouse Experiment

Inoculation and assay procedure

The greenhouse component included separate trials with pepper and tomato. All the procedures were similar except the strains P6 and T4 were used in pepper and tomato. Tomato and pepper plants seedlings were transplanted in 10 cm-diameter pots

in a commercial soil mix Fafard no. 2 (Conrad Fafard, Inc., Agawan, MA.). Each pot received 5 to 10 g of a slow-release fertilizer (Osmocote, 15-9-12; Sierra Chemical Co., Milpitas, CA). The plants were maintained in an air-conditioned greenhouse. Tomato plants at 3-4 weeks of age and pepper plants at 4-5 weeks were sprayed with the chemicals suspensions listed in Table 4-1 using a hand-held pump sprayer. Six plants were spayed per chemical suspension. Control consisted of six plants sprayed with sterile tap water. Once the plants were dry, they were transported to a growth-room for 2 days in which environmental conditions included a temperature of 28°C, a 12-hour light and a 12-hour dark cycle. Using a hand-held pump sprayer, both adaxial and abaxial leaf surfaces were inoculated by spraying until run-off with a bacterial suspension (A_{600} = 0.3), consisting of a 24-hour culture suspended in tap water. All plants were immediately encased in transparent, polyethylene, plastic bags for a 48-h period. The bags were then removed and the plants were returned to the greenhouse for the remainder of the experiment. The treatments were distributed in a randomized complete block design. The experiment was done twice.

Rating and statistical analysis

Fourteen days after inoculation, the plants were rated as disease incidence by counting the number of lesions on the fifth leaf (from bottom to top) on tomato or as disease severity by estimating the percent of leaf area affected by bacterial spot on pepper. Data were transformed either logarithmically in case of number of lesions or using the Horsfall-Barrett scale with pepper data. Statistical analysis was carried using SAS (SAS version 9.1, SAS Institute, Cary, NC). The analysis included variance and a mean separation using the Least Significant difference (LSD).

Results

In *vitro* assays

Tanos alone and its components, as expected, did not have any direct bactericidal effects on the strains T4, P6 and L7 (Tables 4-2, 4-3). In some trials (Table 4-4), bacterial populations increased significantly in comparison to the control. With the strains T4 and P6, Kocide 3000 + Tanos + Manzate 75DF appeared to be the most bactericidal in four of the six trials, whereas Kocide 3000 + Manzate 75DF was the most toxic in two of the six trials. There is also a trend for Tanos to induce a modest increase in the toxicity of Kocide in comparison to Kocide alone. For the strain T4, in all three trials, the combination of Kocide + Tanos was significantly better than Kocide alone; however, no statistical difference was detected in two of three trials with the strain P6 (Table 4-3) and in only one trial, Kocide + Tanos resulted in a larger reduction of bacterial populations than Kocide alone. The addition of Cymoxanil or Famaxadone to Kocide do not appear to increase the toxicity of Kocide with no stastical differences in populations in most of the trials.

Table 4-2. *In vitro* trials with the copper-resistant strain T4 (*Xanthomonas perforans*) after incubation with various chemical compounds

	Trial 1[a]		Trial 2		Trial 3
Tanos	9.38 A	Cymoxanil	9.46 A	Famaxadone	8.92 A
Famaxadone	9.38 A	Famaxadone	9.44 A	Tanos	8.82 A
Cymoxanil	9.27 A	Control	9.34 A	Cymoxanil	8.72 A
Control	9.20 A	Tanos	9.33 A	Control	8.37 A
Kocide	6.42 B	Kocide + Famaxadone	6.95 B	Kocide	3.96 C
Kocide + Famaxadone	6.41 B	Kocide + Cymoxanil	6.75 B	Kocide + Famaxadone	3.82 C
Kocide + Cymoxanil	6.21 B	Kocide	6.51 B	Kocide + Cymoxanil	3.48 D
Kocide + Tanos + Mancozeb	2.16 C	Kocide + Mancozeb	5.53 C	Kocide + Tanos	3.16 E
Kocide + Tanos	1.50 C	Kocide + Tanos	4.59 D	Kocide + Mancozeb	0.29 F
Kocide + Mancozeb	0.00 D	Kocide + Tanos + Mancozeb	1.98 E	Kocide + Tanos + Mancozeb	0.00 G

[a]Data are expressed as Log CFU. Numbers in columns followed by the same letter are not significantly different at P< 0.05 according to the LS Means test.

Table 4-3. *In vitro* trials with the copper-resistant strain P6 (*Xanthomonas euvesicatoria*) after incubation with various chemical compounds

	Trial 1[a]		Trial 2		Trial 3
Famaxadone	9.57 A	Cymoxanil	9.96 A	Control	9.39 A
Cymoxanil	9.55 A	Control	9.95 A	Cymoxanil	9.31 A
Control	9.52 A	Famaxadone	9.93 A	Famaxadone	9.18 A
Tanos	9.48 A	Tanos	9.87 A	Tanos	8.97 A
Kocide + Tanos	6.84 B	Kocide	7.52 B	Kocide	5.77 B
Kocide	6.49 B	Kocide + Famaxadone	7.40 B	Kocide + Cymoxanil	5.54 B
Kocide + Cymoxanil	6.26 BC	Kocide + Tanos	7.29 B	Kocide + Famaxadone	4.76 C
Kocide + Famaxadone	5.69 CD	Kocide + Cymoxanil	7.19 B	Kocide + Tanos	3.18 D
Kocide + Tanos + Mancozeb	5.58 D	Kocide + Mancozeb	2.22 C	Kocide + Mancozeb	0.44 E
Kocide + Mancozeb	1.61 E	Kocide + Tanos + Mancozeb	0.56 D	Kocide + Tanos + Mancozeb	0.00 E

[a]Data are expressed as Log CFU. Numbers in columns followed by the same letter are not significantly different at P< 0.05 according to the LS Means test.

Table 4-4. *In vitro* trials with the copper-sensitive strain L7 (*Xanthomonas campestris* pv. *vitians*) after incubation with various chemical compounds

	Trial 1[a]		Trial 2
Tanos	9.69 A	Tanos	9.54 A
Cymoxanil	9.64 A	Famoxadone	9.49 A
Control	9.56 A	Control	9.45 A
Famaxadone	9.50 A	Cymoxanil	9.44 A
Kocide + Tanos	0.00 B	Kocide	0.00 B
Kocide	0.00 B	Kocide + Famaxadone	0.00 B
Kocide + Cymoxanil	0.00 B	Kocide + Tanos	0.00 B
Kocide + Famaxadone	0.00 B	Kocide + Cymoxanil	0.00 B
Kocide + Tanos + Mancozeb	0.00 B	Kocide + Mancozeb	0.00 B
Kocide + Mancozeb	0.00 B	Kocide + Tanos + Mancozeb	0.00 B

[a]Data are expressed as Log CFU. Numbers in columns followed by the same letter are not significantly different at P< 0.05 according to the LS Means test.

Table 4-5. Numbers of lesions caused by *Xanthomonas perforans* strain T4 on tomato plants treated with chemicals in greenhouse trials

	Trial 1[a]		Trial 2		Trial 3
Famaxadone	2.47 A	Famaxadone	2.64 A	Control	2.86 A
Tanos	2.45 A	Tanos	2.42 AB	Tanos	2.80 A
Cymoxanil	2.35 A	Control	2.37 B	Famaxadone	2.76 AB
Control	2.31 A	Cymoxanil	2.34 B	Cymoxanil	2.56 ABC
Kocide + Famaxadone	1.42 B	Kocide	2.09 C	Kocide	2.52 ABC
Kocide + Cymoxanil	1.41 B	Kocide + Famaxadone	1.94 CD	Kocide + Tanos	2.41 BC
Kocide + Tanos	1.41 B	Kocide + Tanos	1.82 DE	Kocide + Cymoxanil	2.41 BC
Kocide	1.32 BC	Kocide + Cymoxanil	1.66 EF	Kocide + Famaxadone	2.32 C
Kocide + Mancozeb	1.12 C	Kocide + Tanos + Mancozeb	1.61 EF	Kocide + Tanos + Mancozeb	2.27 C
Kocide + Tanos + Mancozeb	1.10 C	Kocide + Mancozeb	1.53 F	Kocide + Mancozeb	2.23 C

[a]Data are expressed as Log of number of lesions on 5th leaf. Numbers in columns followed by the same letter are not significantly different at $P< 0.05$ according to the LS Means test.

Table 4-6. Effects of *Xanthomonas euvesicatoria* strain P6 on pepper plants treated with chemicals in greenhouse trials

	Trial 1[a]		Trial 2		Trial 3
Cymoxanil	10.00 A	Cymoxanil	8.80 A	Tanos	11.67 A
Control	9.83 A	Famaxadone	8.60 A	Cymoxanil	11.00 AB
Famaxadone	9.83 A	Tanos	8.50 A	Famaxadone	10.67 AB
Tanos	9.17 A	Control	8.40 A	Control	10.33 B
Kocide	7.00 B	Kocide + Famaxadone	6.80 B	Kocide + Famaxadone	7.17 C
Kocide + Famaxadone	6.83 B	Kocide	6.50 BC	Kocide	6.50 CD
Kocide + Cymoxanil	6.17 BC	Kocide + Cymoxanil	6.40 BC	Kocide + Cymoxanil	6.00 DE
Kocide + Tanos	5.83 BC	Kocide + Tanos	5.83 C	Kocide + Tanos	5.50 DEF
Kocide + Mancozeb + Tanos	5.00 C	Kocide + Mancozeb	3.67 D	Kocide + Mancozeb	5.17 EF
Kocide + Mancozeb	4.67 C	Kocide + Mancozeb + Tanos	3.50 D	Kocide + Mancozeb + Tanos	4.83 F

[a]Data are expressed as Horsfall Barett rating (HB). Numbers in columns followed by the same letter are not significantly different at $P< 0.05$ according to the LS Means test.

Disease Control on Tomato in the Greenhouse

Plants treated with Tanos, Famaxadone or Cymoxanil did not induce any disease control in all three trials. In the first trial, a similar level of control was obtained between the Kocide alone, and Kocide + Mancozeb or Mancozeb + Tanos + Kocide. The addition of Tanos, Cymoxanil or Famaxadone to Kocide resulted in a similar disease control to Kocide alone. In the second trial, only the combination Kocide + Famaxadone was similar to Kocide alone; the other mixtures that contained Kocide had significantly reduced disease severity. There was a lot of variation in disease control in disease control.

Disease Control on Pepper in the Greenhouse

In the first trial, the disease severity of the untreated plants had a mean rating of 9.83 (HB rating, Table 4-6), which was not significantly different from the means of plants treated with Tanos, Cymoxanil or Famaxadone. A similar pattern was observed for the two other trials. In trials 1 and 3, the mixture Tanos/Kocide resulted in a similar disease control to the standard combination of Kocide/ Manzate. Tanos tends to increase the efficacy of the mixture Kocide/Manzate in two of the trials. In all trials, Kocide alone provided a similar level of control as the combinasion of Kocide with Tanos, Cymoxanil or Famaxadone.

Discussion

In this study, we were interested in dissecting the components of Tanos- Cymoxanil and Famaxadone- in the control of bacterial spot of tomato and pepper, and bacterial leaf spot of lettuce. In Florida, the control of bacterial spot is primarily based on copper-mancozeb sprays. The EBDC can break down in the environment and can generate carcinogenic residue on fruits (US EPA, 1992). The use of EBDCs may be

further regulated on food crops. Therefore, it is desirable to investigate alternative compounds as a potential substitute for mancozeb.

Tanos, Cymoxanil and Famaxadone did not have any inhibitory activity against the *in vitro* growth of *X. perforans, X. euvesicatoria and X. campestris* pv. *vitians*. In some of the trials, the chemicals appeared to promote bacterial growth as previously reported by Roberts et al. (2008). In all trials, greenhouse data reflected the trends shown by Cymoxanil, Famaxadone and Tanos. No disease suppression was observed with plants treated with these chemicals. However, Roberts et al. (2008) reported disease suppression with applications of Famaxadone in greenhouse experiments. Pernezny et al. (2008b) found that mancozeb and Famaxadone/Cymoxanil did not have any inhibitory effects on *X. campestris pv. vitians, X. perforans and X. euvesicatoria in vitro*. They coucluded that the reports of disease suppression with Famaxadone/Cymoxanil (Roberts et al., 2008) might not be due to bactericidal activity but rather due to another mode of action, such as systemic-acquired resistance.

As previously reported by Pernezny et al. (2008b), there is a trend for Tanos to induce a modest synergistic increase in the toxicity of Kocide and Manzate to *X. perforans* and *X. euvesicatoria*. In four of the six *in vitro* trials with the strains *X. euvesicatoria and X perforans*, this reduction was statistically significant (4.2). There is a trend of synergism for the mixture of Tanos and Copper for all *in vitro* trials with the strain *X. perforans* (Table 4-2). However, increased disease control was not obtained with the greenhouseb data since there was no statistical difference between Kocide alone, and Kocide/Tanos for disease control in five of the six trials (Tables 4-5, 4-6). In some instances, the mixture Kocide/Tanos led to similar level of disease control as the

standard mixture of Kocide/Manzate, and Kocide + Tanos + Manzate. Roberts et al. (2008) reported that various mixtures of Famaxadone, Famaxadone + Cymoxanil, mancozeb, and copper, all supressed bacterial spot in comparison to the applications of the copper-mancozeb standard. The use of Famaxadone plus Cymoxanil and mancozeb could possibly reduce the application of copper, which might help reduce the selection of copper-resistant *Xanthomonas* strains and the accumulation of copper in the environment (Roberts et al., 2008).

In vitro and greenhouse trials pointed to copper/mancozeb, including Tanos in some instances, as the best treatment. Addition of mancozeb to copper usually resulted in increased mortality of the pathogen. This might be due to a higher concentration of Cu^{2+} ions in solution (Marco and Stall, 1983). However, Jones et al. (1991) hypothesized that soluble copper may contribute to toxicity against the Cu^{r} strains, but does not appear to be the primary component involved in the toxicity of the copper/mancozeb combination to Cu^{r} strains of *X. c. vesicatoria*. Other workers demonstrated that zinc is involved in the observed control of bacterial spot (Adaskaveg and Hine, 1985). Mancozeb may also chelate copper ions, therefore increasing their availability to certain sites in bacterial cells (Medhekar and Boparai, 1981). If levels of free ions increase, a number of toxic effects can occur in cells (Cooksey, 1994). Copper is a potent inhibitor of photosynthetic electron transport at concentrations higher than 1 µM (Mohanty et al., 1989).

In one trial with a strain of *X. perforans*, T4, (Table 4-5), no difference was obtained with Cymoxanil and all the treatments that contained copper, including the standard copper/mancozeb mixture. No significant difference was obtained between the

untreated plants and those treated with Cymoxanil. Pernezny et al. (2008b) reported that the addition of mancozeb to copper did not induce a sufficient reduction of bacterial populations for a strain of *X*. *euvesicatoria*. Populations were still above $1x10^6$ CFU/mL after a 2-hour exposure *in vitro*. These observations support the suboptimal performance of the standard copper/mancozeb mixture under optimal conditions for disease development (Jones and Jones, 1985).

CHAPTER 5
INFLUENCE OF TANOS AND ITS COMPONENTS IN THE POPULATION DYNAMICS ON THE LEAVES

Microbial population dynamics on leaves are related to four processes: immigration, emigration, growth and death (Kinkel, 1997). Several patterns have been generally reported for leaf surface populations: a) aggregation of phyllosphere microbial populations within individual leaves, b) variability among leaves and among plants, c) increase over the growing season, d) high variability over short period of time, associated with specific environmental events (e.g., rainstorms), and e) existence of seasonal patterns among phyllosphere populations (Kinkel, 1997).

The terms epiphytic, phylloplane, resident and leaf-surface bacteria are interchangeably used in the literature (Beattie and Lindow, 1995; Hirano and Upper, 1983). It is generally admitted that epiphytic bacteria are able to live (multiply) on plant surfaces (Hirano and Upper, 1983; Leben, 1965). Leben (1963, 1965) used the terms “residents” and “casuals” to differentiate bacteria that can multiply on leaf surfaces from those that may reach the leaves by chance but can not multiply. From a functional perspective, epiphytic bacteria are considered those that can be removed from above ground plants parts by washing (Hirano and Upper, 1983). In contrast, endophytic bacteria are considered to be those that can live in the leaf intercellular spaces, substomatal cavities, or vascular tissues and have been functionally defined as those bacteria that remain after the removal of the epiphytic bacteria (Beatie and Lindow, 1995) Both the surfaces and internal regions of the leaves can be colonized by foliar pathogens (Hirano and Upper, 1990; Leben, 1965) and active exchange occurs between the internal and external population (Bashan et al., 1981). Foliar pathogens can have access to internal leaf tissues from the surface (Beattie and Lindow, 1995).

Surfaces structures, such as stomata (Gitaitis et al., 1981), hydathodes (Bretschneider et al., 1989) have been reported as entry sites for many foliar pathogens. Internal population can egress onto the surface. The formation of lesions may increase the amount of cells that egress and subsequently spread the pathogen on the leaf surface (Beattie and Lindow, 1995).

Epiphytic phytopathogenic bacteria can provide inoculum for disease development and for spread to the surface of other plants and plant parts (Hirano and Upper, 1983; Beattie and Lindow, 1995). In a given pathosystem, each epiphytic phytopathogenic bacterium has a finite and extremely low probability of inducing disease. Subsequently, when resident populations are sufficiently high on individual leaves, the probability of causing disease is greater under favorable conditions (Hirano and Upper, 1883). Large epiphytic populations have been associated with time of disease onset and with increased levels of disease (Beattie and Lindow,1995). Under field conditions, detection of disease was always observed at a population level of at least 5×10^6 cells per navy bean leaflet of either *X*. *campestris* pv*. phaseoli* or X*. campestris* pv*. phaseoli var. fuscans* (Weller and Saettler, 1980). For some pathosystems, including brown spot disease of beans (Lindemann et al.1984) and halo blight of oats (Hirano et al. 1981), a quantitative relationship has been established between epiphytic population size and probability of disease occurrence. Increases in resident populations of *P. syringae* pv*. tomato* result in higher incidence of bacterial speck on tomato with a 10- to 12-day lag required for infection and symptom expression (Smitley and McCarter, 1982). Studying the role of *hrp* genes in the fitness of *P. syringae* on beans, Hirano et al. (1997; 1999)

found that an intact type III secretion system is required for the growth, and possibly the survival of *P. syringae* in the phyllosphere.

It is generally accepted that bacteria must gain access to the internal tissues and establish large endophytic populations for successful infection. Therefore, the internal populations, not the epiphytic populations, are essential for disease development. However, there is a strong correlation between high epiphytic population sizes and high probability of disease occurrence in some foliar diseases. One reason for this is that increased epiphytic populations potentially result in higher endophytic population sizes (Beattie and Lindow, 1995). However, it was been shown that large shoot surface populations of phytopathogens can exist in the absence of disease (Hirano and Upper, 1983, Leben, 1965). Thus, large resident populations of a phytopathogen may increase the probability of endophytic populations, but their presence does not ensure the development of large endophytic populations that results in disease. Based on infectivity titration experiments, a bacterial concentration of 10^4 cfu/ml was enough to initiate disease in compatible host/pathogen inoculations (Robinson et al., 2006). In the presence of large epiphytic population size, the extent of ingress, which relies on the number of entry sites available (Ramos and Volin, 1987) and environmental conditions (Daub and Hagerdorn, 1979), is a major factor that influences disease induction. The number of entry sites is influenced by host genotype, leaf age, position on leaf surfaces and wounds (Ramos and Volin, 1987; Beattie and Lindow, 1995).

Jones et al. (1991) reported that copper and a mixture of copper and mancozeb reduced the epiphytic population of *X*. *campestris* pv. *vesicatoria* in comparison to the untreated plants. A positive correlation, between epiphytic populations and disease

severity, was also found. In one test, Pernezny and Collins (1997) found that copper sprays reduced *X. campestris* pv*. vesicatoria* populations on pepper leaflets in 99% in comparison to a reduction of 51 % in buds. This study was undertaken to determine the effects of Tanos and its components alone in combination with copper and mancozeb, in some instances, on the population dynamics of Cu^r strains of *X. perforans* and *X. euvesicatoria* on tomato leaves and pepper leaflets.

Materials and Methods

Strains and Inoculum

Rifampicin-resistant P6 and T4 strains were selected as follow : 25 uL aliquots of 24-h-old bacteria culture, grown in Nutrient Broth (Laboratories Difco), were spread on Nutrient Agar (NA, Laboratories Difco) plates amended with rifampicin (25 µg/mL). Plates were incubated for 3 days at ^{0}C. Rifampicin-resistant colonies (Rif T4; Rif P6) were selected. RifT4 or RifP6 strains were grown for 24-h on NA amended with rifampicin, and then flooded with a solution of 0.01 M $MgS0_4$. Suspensions were adjusted to an optical density of 0.30 at 600 nm with a spectrophotometer. The inoculation of the plants, the time in the growth chamber and the application of the chemical suspensions were as previously described for the greenhouse component in the previous chapter.

Leaf Sampling and Assay Procedure

Three inoculated leaflets per treatment were randomly sampled 0, 2, 4, 6, 8, 10 and 12 days after inoculation, and then at days. Each leaflet was placed in a 50 mL tube weighed and then mixed with a volume of 10 mL of peptone buffer per gram of tissue. The buffer contains (per liter) 5.3 g of KH_2PO_4, 8.6 g of Na_2HPO_4, and 1 g of bacto peptone (McGuire et al.1986). Tubes were shaken on a rotary shaker at 200 rpm for 45

min. Serial 10-fold dilutions were made in of 0.01 M $MgS0_4$. A 50-µl aliquot of different serial dilutions was plated onto each of three plates of Nutrient Agar amended with rifampicin. After incubation at 28^0C for 3 days, typical colonies of *X*. *perforans* or *X*. *euvesivatoria* were counted. Statistical analysis was carried out with $\log_{10}$ transformed data. Data were expressed as $\log_{10}$ CFU/g of tissue. The analysis included variance and a mean separation using the Least Significant difference (LSD).

Results

Epiphytic populations of *X. euvesicatoria* show a variable pattern among the treatments in both trials. In one trial with a strain of *Xanthomonas euvesicatoria* (Table 5-2) and one trial with *Xanthomonas perforans* (Table 5-3), some statistical differences ($P \leq 0.05$) were obtained among some treatments in some sampling days (Day 0, Day 2, Day 4, and Day 6) whereas no statistical difference was obtained for the remainder of the experiment. Generally, the addition of Tanos, and its components to Kocide in some instances, did not reduce epiphytic populations at significant level in comparison to Kocide alone, and the control in some days. The general trend is that the populations in different treatments are the same over the period during which the samplings were carried out. After inoculation (Day 0), the epiphytic populations of *X. euvesicatoria* of all plants treated with copper and Famaxadone were similar, whereas these populations were significantly lower than those of untreated plants, or plants treated with Cymoxanil, or Tanos. In samplings days 2 and 4, epiphytic populations on leaflets treated with Tanos were higher than on untreated control plants.

Table 5-1. Populations dynamics of a strain of *X. euvesicatoria* on pepper leaflets

Day 0[a]		Day 2		Day 4		Day 6		Day 8		Day 12	
Famaxadone	9.73 A	Control	9.86 A	Kocide + Tanos	5.00 A	Cymoxanil	6,73 A	Kocide + Tanos + Mancozeb	10.98 A	Kocide	9.36 A
Cymoxanil	9.66 A	Cymoxanil	9.76 A	Cymoxanil	4.82 A	Famaxadone	6.39 AB	Cymoxanil	10.94 AB	Tanos	9.12 A
Control	9.65 A	Tanos	9.75 A	Tanos	4.44 AB	Kocide + Famaxadone	6.34 AB	Kocide + Famaxadone	10.94 AB	Famaxadone	9.01 A
Tanos	9.62 A	Kocide	8.71 B	Famaxadone	4.43 AB	Control	6.26 AB	Control	10.92 AB	Kocide + Cymoxanil	8.33 A
Kocide + Famaxadone	8.06 B	Kocide + Tanos	8.40 BC	Control	4.23 AB	Kocide	5.99 ABC	Tanos	10.92 AB	Kocide + Famaxadone	8.32 A
Kocide	7.87 B	Famaxadone	8.30 BC	Kocide + Famaxadone	3.66 AB	Kocide + Cymoxanil	5.77 ABC	Kocide + Cymoxanil	10.91 AB	Control	8.16 A
Kocide + Tanos	7.63 BC	Kocide + Famaxadone	8.25 BC	Kocide + Mancozeb	3.55 AB	Tanos	5.35 ABC	Kocide + Tanos	10.91 AB	Kocide + Tanos + Mancozeb	8.05 A
Kocide + Cymoxanil	7.17 C	Kocide + Cymoxanil	8.06 C	Kocide	3.49 AB	Kocide + Tanos	5.28 ABC	Famaxadone	10.89 AB	Kocide + Tanos	7.82 A
Kocide + Tanos + Mancozeb	6.52 D	Kocide + Mancozeb	5.91 D	Kocide + Cymoxanil	2.94 B	Kocide + Tanos + Mancozeb	4.32 BC	Kocide + Mancozeb	10.88 B	Kocide + Mancozeb	7.56 A
Kocide + Mancozeb	5.58 E	Kocide + Tanos + Mancozeb	5.81 D	Kocide + Tanos + Mancozeb	2.88 B	Kocide + Mancozeb	3.79 C	Kocide + Tanos + Mancozeb	10.86 B	Cymoxanil	7.54 A

[a]Data are expressed as Log CFU/g. Numbers in columns followed by the same letter are not significantly different at P< 0.05 according to the LS Means test.

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Table 5-2. Populations dynamics of a strain of *X. euvesicatoria* on pepper leaflets

Day 0 [a]		Day 2		Day 4		Day 6		Day 8		Day 10		Day 12	
Famaxadone	8.08 A	Tanos	7.94 A	Tanos	6.92 A	Tanos	7.50 A	Tanos	8.89 A	Tanos	11.98 A	Control	11.19 A
Control	7.61 A	Cymoxanil	7.00 AB	Famaxadone	6.18 AB	Famaxadone	6.76 AB	Control	8.15 AB	Control	10.25 AB	Cymoxanil	10.36 AB
Cymoxanil	6.81 AB	Control	6.83 AB	Cymoxanil	5.83 AB	Cymoxanil	5.86 ABC	Famaxadone	7.72 ABC	Kocide + Cymoxanil	9.50 AB	Tanos	10.2 AB
Tanos	6.79 AB	Famaxadone	6.71 ABC	Control	5.53 ABC	Kocide + Famaxadone	5.81 ABC	Cymoxanil	7.69 ABC	Famaxadone	8.38 BC	Famaxadone	10.02 AB
Kocide	5.81 BC	Kocide	5.96 BDC	Kocide	5.45 BC	Kocide + Tanos	5.05 BCD	Kocide	7.40 ABCD	Cymoxanil	8.38 BC	Kocide + Cymoxanil	9.14 ABC
Kocide + Famaxadone	5.61 BCD	Kocide + Tanos	5.53 BDC	Kocide + Famaxadone	4.74 BDC	Control	5.04 BDC	Kocide + Cymoxanil	6.70 ABCD	Kocide + Famaxadone	7.85 BC	Kocide	7.69 BCD
Kocide + Tanos + Mancozeb	5.09 BCD	Kocide + Tanos + Mancozeb	5.47 BDC	Kocide + Tanos	4.14 CDE	Kocide + Cymoxanil	4.94 BDC	Kocide + Tanos + Mancozeb	6.57 ABCD	Kocide	7.63 BC	Kocide + Tanos	6.77 CD
Kocide + Cymoxanil	4.46 CD	Kocide + Famaxadone	5.23 DC	Kocide + Cymoxanil	3.58 DE	Kocide	4.87 BDC	Kocide + Famaxadone	6.18 BCD	Tanos + Kocide	6.43 CD	Kocide + Famaxadone	6.18 D
Kocide + Tanos	4.83 CD	Kocide + Mancozeb	4.72 D	Kocide + Tanos + Mancozeb	3.33 ED	Kocide + Tanos + Mancozeb	4.28 CD	Kocide + Mancozeb	5.28 CD	Kocide + Tanos + Mancozeb	5.64 CD	Kocide + Mancozeb	5.14 D
Kocide + Mancozeb	4.00 D	Kocide + Cymoxanil	4.71 D	Kocide + Mancozeb	3.25 E	Kocide + Mancozeb	3.61 D	Kocide + Tanos	4.96 D	Kocide + Mancozeb	4.49 D	Kocide + Tanos + Mancozeb	5.14 D

[a]Data are expressed as Log CFU/g. Numbers in columns followed by the same letter are not significantly different at P< 0.05 according to the LS Means test.

Table 5-3. Populations dynamics of a strain of *X. perforans* on tomato leaves

	Day 0 [a]		Day 2		Day 4		Day 6		Day 8		Day 10		Day 12
Cymoxanil	8.77 A	Control	10.64 A	Control	9.07 A	Cymoxanil	9.34 A	Control	10.60 A	Control	10.40 A	Famaxadone	11.47 A
Tanos	8.49 A	Tanos	9.50 AB	Famaxadone	8.93 A	Famaxadone	9.27 A	Tanos	10.37 AB	Famaxadone	10.07 A	Tanos	10.43 AB
Control	8.47 A	Famaxadone	9.47 AB	Tanos	8.88 AB	Control	9.17 A	Cymoxanil	9.40 ABC	Cymoxanil	9.83 AB	Control	9.97 BC
Famaxadone	8.46 A	Cymoxanil	9.10 B	Cymoxanil	8.47 AB	Tanos	9.13 A	Kocide	8.73 BCD	Tanos	9.00 ABC	Cymoxanil	9.23 CD
Kocide	7.76 AB	Kocide	7.53 C	Kocide + Cymoxanil	7.50 BC	Kocide	7.33 B	Famaxadone	8.70 BCD	Kocide	8.37 BCD	Kocide	8.13 DE
Kocide + Tanos	7.21 BC	Kocide + Famaxadone	7.07 CD	Kocide + Famaxadone	7.03 DC	Kocide + Famaxadone	7.30 B	Kocide + Cymoxanil	8.43 CDE	Kocide + Tanos	8.33 BCD	Kocide + Cymoxanil	7.93 E
Kocide + Famaxadone	6.73 BCD	Kocide + Tanos + Mancozeb	6.43 CD	Kocide	6.80 CD	Kocide + Cymoxanil	7.13 B	Kocide + Tanos	8.03 CDE	Kocide + Cymoxanil	8.27 BCD	Kocide + Famaxadone	7.90 E
Kocide + Tanos + Mancozeb	6.19 CD	Kocide + Tanos	6.33 CD	Kocide + Tanos	6.37 CDE	Kocide + Tanos + Mancozeb	6.73 B	Kocide + Famaxadone	7.53 DE	Kocide + Famaxadone	8.20 CD	Kocide + Tanos	7.60 EF
Kocide + Cymoxanil	6.17 CD	Kocide + Cymoxanil	6.27 CD	Kocide + Mancozeb	5.77 DE	Kocide + Tanos	6.37 BC	Kocide + Tanos + Mancozeb	7.00 E	Kocide + Mancozeb	7.40 CD	Kocide + Tanos + Mancozeb	6.60 FG
Kocide + Mancozeb	5.66 D	Kocide + Mancozeb	5.83 D	Kocide + Tanos + Mancozeb	5.10 E	Kocide + Mancozeb	5.40 C	Kocide + Mancozeb	6.77 E	Kocide + Tanos + Mancozeb	7.17 D	Kocide + Mancozeb	6.20 G

[a]Data are expressed as Log CFU/g. Numbers in columns followed by the same letter are not significantly different at P< 0.05 according to the LS Means test.

As in trial 1 for *X. euvesicatoria*, a similar trend of similar treatments was observed over time in trial 2. Specific reductions of populations could be observed during a giving sampling day, but such differences were not consistent over time, except the combination of copper/mancozeb in relation to the untreated plants. The copper and mancozeb combination consistently reduced epiphytic populations of *X. euvesicatoria* compared to those of the control (Table 5.2) during all the sampling days (exception: day 6). All plants treated with Kocide and another chemical generally presented similar populations than plants treated with Kocide alone. The addition of Tanos to Copper seems to promote modest bactericidal activity in the reduction of epiphytic populations since plants treated with this mixture presented the lowest epiphytic populations – although at levels no statistically different- in most of the sampling days. However, the addition of Tanos did not appear to increase the bactericidal property of the standard mixture of copper/mancozeb since plants treated with copper/mancozeb generally presented lower epiphytic populations than plants treated with Tanos + copper + mancozeb in most of the samplings days.

As observed in trials with *X. euvesicatoria*, the population dynamics of *X. perforans* show a variable pattern among the treatments (Table 5.3). The treatments that contain copper generally induced a similar level of control of epiphytic bacterial populations although population sizes varied greatly among the different treatments with copper. Moreover, the mixture Kocide / Mancozeb tends to be the best treatment in terms of reduction of epiphytic populations in comparison to the untreated plants. The addition of Tanos to Copper seems to have a modest effect since in most sample times,

plants treated with Kocide/Tanos generally have lower populations than those treated with kocide alone.

Discussion

Epiphytic bacteria are able to multiply on plant surfaces (Hirano and Upper, 1983). Large epiphytic populations have been associated with time of disease onset and with disease progression (Beattie and Lindow,1995). For foliar pathogens, like xanthomonads associated with bacterial spot of pepper and tomato, foliar application of chemicals can modify the populations dynamics on the leaves. The effects of copper-based bactericides on phyllosphere populations have been reported in some studies. (Scheck and Pscheidt,1998; Pernezny and Collins, 1997; Jones et al. 1991). The aim of this study was to evaluate the effects of Tanos, Cymoxanil and Famaxadone alone, and in combination with Kocide and in some instances, mancozeb, on the epiphytic populations of a strain of *X. euvesicatoria* and a strain of *X. perforans*.

The general trend is that many treatments were similar in a given sampling period and over time. In both trials with a strain of *X. euvesicatoria*, P6, all treatments that contain Kocide generally did not differ significantly over time although population sizes vary among them in a given sampling day. Specifically, the addition of Tanos, either to copper or to copper/mancozeb, did not result in better bactericidal activity in terms of reduction in phyllosphere populations.

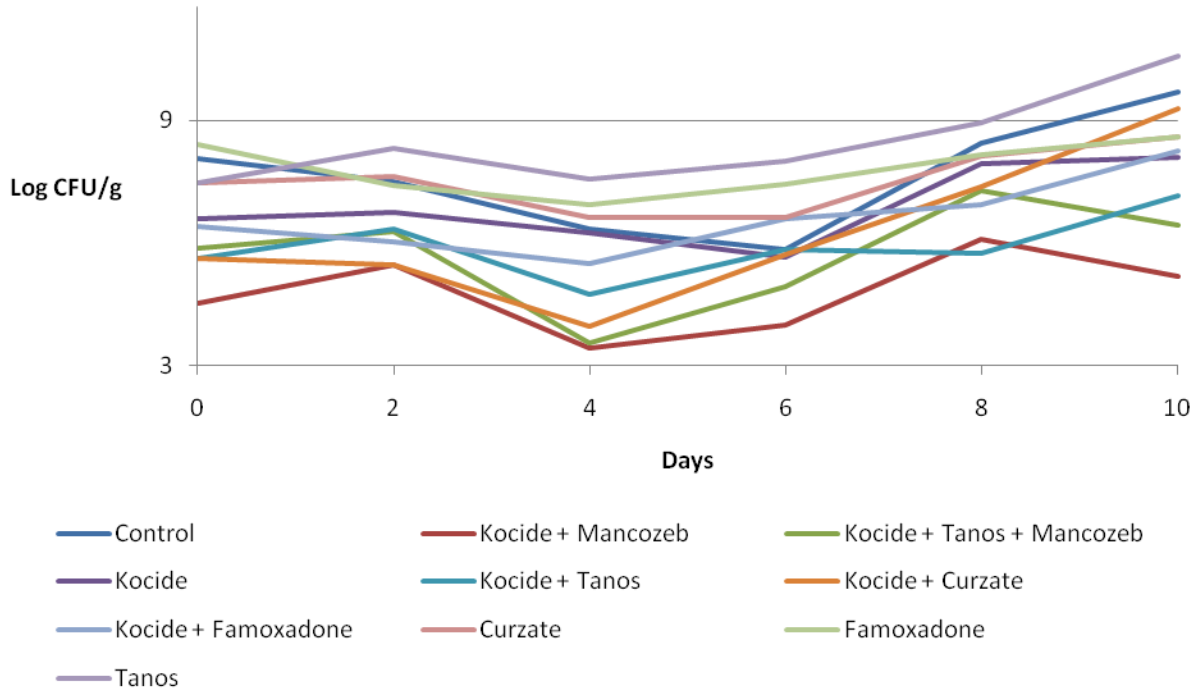

Figure 5-1. Population dynamics of strain of *X. euvesicatoria* on pepper leaflets,Trial 1

In trial 1 with a strain of *X. euvesicatoria*, the general trend is that all treatments were similar over time. Significant reductions of phyllosphere populations among some treatments during the first sampling days were not consistent over time. In this trial, the treatments that contain copper, including the standard copper/mancozeb, did not reduce the phyllosphere populations although plants treated with the standard mixture presented the lowest populations (no statistical difference) in most of the sampling days.

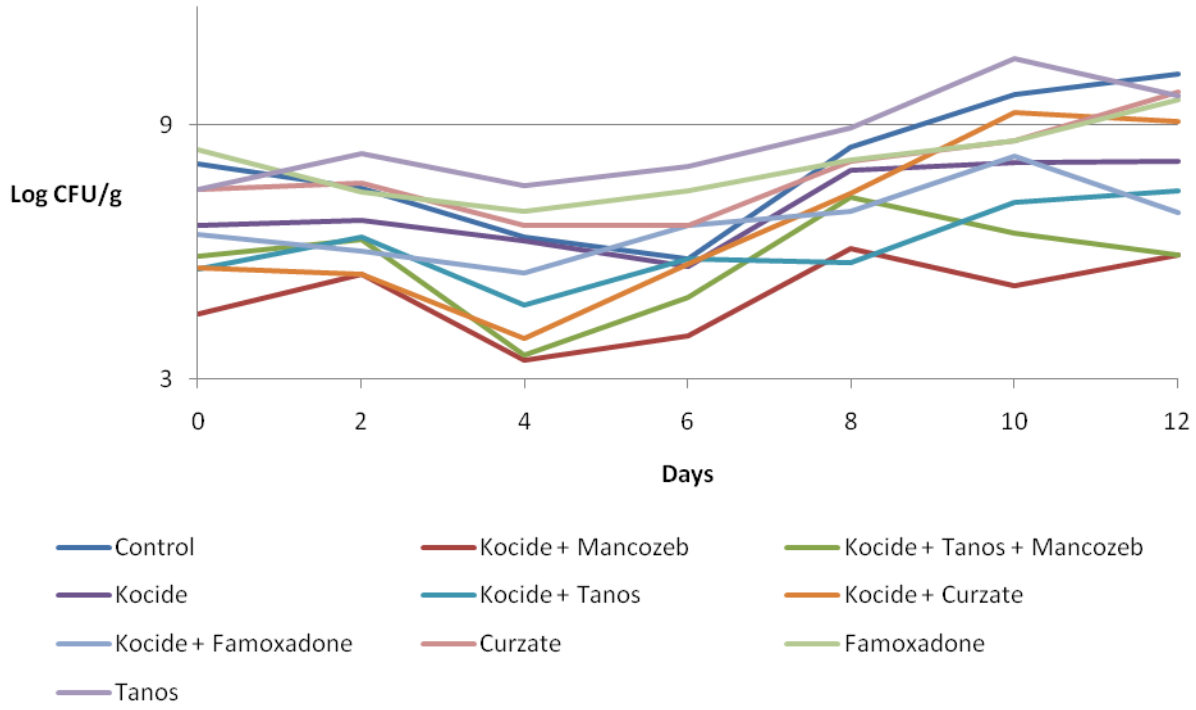

Figure 5-2. Population dynamics of a strain of *X*. *euvesicatoria* on pepper leaflets, Trial 2

In one trial with a strain of *X. euvesicatoria* (Table 5-2 and Figure 5-2), and another one with a strain of *X. perforans* (Table 5-3), the copper/mancozeb mixture appears to be the best treatment over time in terms of reductions in phyllosphere populations. In contrast with the trial 1 with *X. euvesicatoria* (Table 5-1 and Figure 5-1), the standard mixture consistently reduced the pyllosphere populations in comparison to the untreated plants. In a greenhouse study where a copper-resistant strain of *X.c.vesicatoria* was applied to tomato foliage, plants treated with copper or with copper and mancozeb mixture resulted in lower bacterial populations than untreated plants (Jones et al., 1991). Lower bacterial populations on the leaf may reduce initial inoculum, and consequently, affect bacterial spot epidemics (Pernezny and Collins, 1997). A quantitative relationship has been established between epiphytic population size and

probability of disease occurrence for some pathosystems (Lindemann et al. 1984; Hirano et al. 1981). Lindemann et al. (1984) demonstrated that a threshold of 10^4 cfu per gram of leaflet tissue was a good predictor of brown spot incidence on snap beans, caused by *Pseudomonas syringae* pv. *syringae*. Very high populations of this pathogen constituted a more reliable predictor of disease severity than for disease incidence.

Leaf surface chemistry may be an important factor in the interaction between copper bactericides and copper-resistant strains. Copper sprays applied to leaves exist primarily as insoluble deposits of copper salts (Menkissoglu, and Lindow, 1991b). Components of leachates on the tomato leaf surface may modify bacteria sensitivity to copper (Jones et al. 1991). Binding of copper to environmental constituents affects the biological availability of copper and can reduce the toxicity of copper towards microorganisms (Gadd and Griffith, 1978). *In vitro* studies showed that the toxicity of Cu^{2+} to copper-sensitive and copper-tolerant strains of *Pseudomonas syringae* was reduced in the presence of organic compounds including glucose, fructose, sucrose, succinate and citrate (Menkissoglu and Lindow ,1991a). Field trials demonstrated that the concentration of soluble and complexed forms of copper was abundant on navel orange and beans leaves following spray application, but had no significant toxicity towards strains of *P. syringae* (Menkissoglu and Lindow, 1991b). The leachates on pepper and tomato may interact with copper, and therefore could disminish the amount of soluble copper and Cu^{2+}, which might result in less toxicity towards the epiphytic populations of *X. perforans and X. euvesicatoria* on tomato and pepper leaflets respectively.

CHAPTER 6
CONCLUSIONS

Tank mixes of pesticides are often used in plant disease control. In this study, we evaluated Tanos ands its components- Cymoxanil and Famaxadone-, in association with copper, in the control of bacterial spot of tomato and pepper, and bacterial leaf spot of lettuce. Pesticides are combined in order to widen the spectrum of biological activity, to delay the selection of resistant strains and to exploit synergistic interactions (Gisi et al., 1985).

In vitro studies show a synergistic action between copper and Tanos against the *in vitro* growth of a strain of *Xanthomonas perforans*. It was also found that Tanos tended to induce a modest increase in the toxicity of Kocide and Mancozeb to *X. perforans* and *X. euvesicatoria*. It appears that both components of Tanos, Cymoxanil and Famaxadone, are required for synergistic action since the addition of Cymoxanil or Famaxadone to copper did not induce significant reduction of bacterial populations in comparison to copper alone. Tanos, and its components do not have any inhibitory activity against the *in vitro* growth of the pathogens evaluated. In contrast, Tanos appears to promote bacterial growth as previously reported by Robert et al. (2008). Therefore, the mechanism by which this growth occurs could be desirable to elucidate.

There was a lot of variation in disease control in greenhouse studies. Plants treated with the standard mixture copper/Mancozeb, including Tanos in some instances, tended to have a lower disease incidence. But, no statistical difference was obtained in most the trials between the copper/mancozeb and other treatments that contain copper. Various mixtures of Famaxadone + Cymoxanil, mancozeb and copper were reported to induce a similar disease suppression as copper/mancozeb. The use of Famaxadone +

Cymoxanil could possibly reduce the application of copper, which might help reduce the selection of copper-resistant *Xanthomonas* strains and the accumulation of copper in the environment (Roberts et al. 2008).

A variable pattern was also obtained in the study related to the populations dynamics of *X. perforans*, and *X. euvesicatoria* on tomato and pepper leaflets. High variability of epiphytic populations could be observed over a short period of time (Kinkel, 1997). All treatments that contain copper generally did not differ at stastistical level in a given sampling period. In one trial with a strain of *X. perforans,* the standard mixture copper/mancozeb did consistently reduce the pyllosphere populations in each sampling period in comparison to the control. The addition of Tanos to the copper/mancozeb mixture did not appear to increase bacteridal activity of this mixture to epiphytic populations.

The rates of chemicals that were used in this study are recommended dosis for field applications. However, concentration of fungicides can be a major component in synergistic interactions. Synergistic interactions occured when oxadixyl, mancozeb and Cymoxanil, were mixed in different concentrations against sensitive strains of *Phytophthora infestans* in vivo (Gisi et al. 1985). Wadley's method of assessing synergism uses an approach of different concentrations to construct the dose responses of fungicides in mixture. Equally effective concentrations, EC-values, for different levels of control, are computed (Gisi et al. 1985, Levy et al., 1986). Therefore, the synergistic trend observed in some in *vitro trials* with strains of *X. euvesicatoria and X. perforans* could be further supported by studying different concentrations of the chemicals involved in the synergic interactions.

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BIOGRAPHICAL SKETCH

Joubert Fayette was born in Haiti. He graduated from the “Collège Les Normaliens Réunis” High School in 2002. He attended “Faculté d’Agronomie et de Médecine Vétérinaire”, State University of Haiti, from 2002 to 2003. Then, he moved to Costa Rica where he earned his Bachelor of Science degree in agronomical engeneering at EARTH (Escuela de Agricultura de la Región Tropical Húmeda) University in December 2007. In 2006, he did an undergraduate internship at Southwest Florida Research and Education Center, University of Florida. During his undergraduate studies, he evaluated several plants extracts against the *in vitro* growth of *Xanthomonas perforans*, *Phytophthora capsici* and *Colletotrichum gloesporioides.* He attended the graduate program at the University of Florida, College of Agricultural and Life Sciences, Department of Plant Pathology, from August 2008 to August 2010. He conducted a research project that was based on the evaluation of Tanos and its components, in association with copper, and mancozeb in some instances, in the management of bacterial spot of tomato and pepper, and bacterial leaf spot of lettuce.