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Evaluating the Efficacy of Commercial Products for Management of Bacterial Leaf Spot on Lettuce

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Abstract

The efficacy of Serenade, Maneb, Cuprofix, and mixtures of these products were evaluated for efficacy in reducing disease incidence and severity of bacterial leaf spot of lettuce caused by *Xanthomonas campestris* pv. *vitians* in six trials at two locations between 2001 and 2003. Additionally, Messenger, elemental copper, Quadris, and Actigard were evaluated in at least two of these trials. Mixtures of Serenade and Cuprofix reduced disease incidence in three of six experiments and reduced disease severity in a fourth experiment. Treatment with the Maneb and Cuprofix mixture was less effective but resulted in significantly less disease and lower severity in single experiments. These results indicate that a mixture of Serenade and Cuprofix may be a useful addition to an integrated bacterial leaf spot disease management strategy but this treatment may be too variable to justify it as a stand-alone treatment.

Introduction

Bacterial leaf spot of lettuce (*Lactuca sativa* L.) caused by *Xanthomonas campestris* pv. *vitians* Dye 1978 (6) was first described in the United States by Nellie Brown in 1918 from diseased lettuce in South Carolina and Virginia. The disease was first reported in California in 1964 (14). It has since been reported from the major lettuce growing regions worldwide (16) probably due to contamination of seed (13,14,17). In addition to survival on seed, the pathogen can survive in fields in plant debris and on leaves of symptomless weeds during fallow periods between lettuce crops (3).

The pathogen causes small angular leaf spots, which are initially water-soaked and later become necrotic (brown to black) and papery. The leaf spots can coalesce, forming large necrotic regions. The symptoms reduce the quality of the lettuce as well as increase the potential of postharvest losses. The pathogen has a relatively minor impact on the California lettuce industry except when environmental conditions are conducive to disease, generally during cool humid conditions created by rain and fog or overhead sprinkler irrigation. Over the last 13 years, reports of significant outbreaks have increased in the Central Coast region of California (3,17), the most important lettuce production area in California and therefore the U.S. (2).

Host resistance is an effective tool to combat bacterial diseases of plants. Although all lettuce types appear to be susceptible to this pathogen (7), two green leaf cultivars, 'Waldmann's' and 'Grand Rapids' (7), one red leaf cultivar, 'Redine' (13), and one cos cultivar, 'Little Gem' (10), have been shown to be resistant to bacterial leaf spot. However, the nature of resistance in these cultivars has not been described.

Bactericides can control other bacterial diseases of vegetables (15). For bacterial leaf spot of lettuce, bactericides have been evaluated as disinfectants for seed and applications for disease control in transplant production (7,12,13). Treatments with copper-based compounds reduced disease severity on lettuce transplants evaluated under standard transplant production conditions in Québec (7).

Serenade and other biological pesticides containing *Bacillus* sp. as the active ingredients are thought to control pathogens in part due to the activation of the host plant's defense system (5). This may be one reason that the list of diseases controlled according to the Serenade label includes diseases caused by a wide range of pathogens on an array of hosts. Interestingly, another biopesticide, Messenger, may also function through stimulation of the plant's defenses (11). Although there are no peer-reviewed publications demonstrating the efficacy of Serenade against bacterial plant pathogens, one scientific abstract (9) reported that this product reduced diseases caused by other *X. campestris* pathovars. Commercial biopesticides have not been evaluated for management of bacterial leaf spot on lettuce.

The purpose of this study was to evaluate biological treatments and chemical bactericides for their efficacy as field applications for the management of bacterial leaf spot of lettuce grown under coastal California production conditions.

Efficacy of Materials for Control of Bacterial Leaf Spot of Lettuce.

Six field experiments were established at two locations during the spring or fall from Spring 2001 to Fall 2003. The susceptible iceberg cultivar 'Vista Verde' was planted in two rows on 40-inch beds in either early April or late August and thinned 3 to 4 weeks later to 12 inches. Plots were 10 ft long and 6.5 ft (2 beds) wide, containing approximately 20 plants per plot. A 5-ft gap was left between each plot to minimize drift from applications. Experiments were designed and analyzed as completely randomized blocks with four replications per treatment.

A mixture of three *Xanthomonas campestris* pv. *vitians* strains Xav 98-12 (4), BS339 and BS347 were used in all experiments. The bacteria were originally isolated from diseased lettuce plants in the Salinas Valley, California. Bacteria were stored at –112°F in a 50:50 solution of glycerol and nutrient broth (NB, Difco Laboratories, Detroit, MI) until needed. For all experiments, the strains were grown in individual flasks of NB on a shaker at 200 rpm for 48 h. Cultures were centrifuged for 10 min at 7,000 rpm, the supernatant was decanted, the bacterial pellets were re-suspended in sterile distilled H_2O, and the suspension was centrifuged again as described. The resultant bacterial pellets were adjusted to approximately 3.79×10^{11} cfu/gallon (1×10^{8} cfu/ml) spectrophotometrically and then combined in equal parts. Bacterial concentrations were confirmed by dilution plating. Approximately 0.03 fluid oz of inoculum was applied to each plant using a CO_2 pressurized handheld sprayer (Model D, R & D Sprayers, Opelousas, LA). To enhance disease development, 5 min of overhead irrigation was applied immediately prior to bacterial inoculations.

Within 3 days after the first bacterial application, the first treatments were applied (Table 1). A surfactant, Silwet L-77 (Helena Chemical, Collierville, TN), was added to each material at application (0.025% v/v). Each treatment was applied, using a CO_2 pressurized handheld sprayer set at approximately 35 psi, with a 4-nozzle hand-held spray boom (19 inch spacing), and Teejet 8005 flat fan nozzles. Materials were applied in the equivalent of approximately 85 gal of water per acre. Applications were made weekly for 3 weeks, and then once every 2 weeks until harvest, resulting in 4 to 6 applications. Actually, four applications were probably sufficient since the fifth and sixth applications occurred just before the final evaluation and harvest. Compounds in mixtures were applied at the same rates as stand alone applications of those compounds.

Table 1. Materials evaluated for their efficacy to reduce incidence and disease severity of bacterial leaf spot of lettuce.

Commercial name	**Active ingredient**	**Manufacturer**	**Rate applied[y]**
Actigard-50 WDG[z]	1,2,3-Benzothiadiazole-7-carbothioic acid S-methyl ester	Syngenta, Greensboro, NC	3/4 oz/acre
Copper WP	Copper sulfate	CP Chemicals, Inc., Fort Lee, NJ	4 lbs/acre
Cuprofix WP	Basic copper sulfate	Cerexagri, King of Prussia, PA	3.75 lbs/acre
Maneb WDG	Manganese ethylene bis-dithiocarbamate	Cerexagri, King of Prussia, PA	2 lbs/acre
Messenger WDG	Harpin protein	Eden Bioscience, Bothell, WA	8 oz/acre
Quadris Flowable	Azoxystrobin; methyl (*E*)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate	Syngenta, Greensboro, NC	15.4 oz/acre
Serenade WP	*Bacillus subtilus* strain QST 713	AgraQuest, Davis, CA	6 lbs/acre

[y] Rate applied refers to one application and not the over-all amount applied.

[z] WP, wetable powder; WDG, wetable dry granule.

Disease incidence and disease severity were assessed in each plot one week before harvest. Disease incidence was determined by calculating the proportion of diseased plants in each treatment replicate. Disease severity was evaluated by rating the most severely damaged area on the plant on a scale of 0 to 5 (plants with no visible symptoms = 0; a few individual lesions = 1; many individual lesions = 2; small patches of coalesced lesions = 3; medium sized patches of coalesced lesions = 4; and large patches of coalesced lesions = 5) (Fig. 1). After checking for normality and equal variances among treatments, an analysis of variance was conducted and, when appropriate, means were separated according to the Tukey-Kramer HSD using JMP (Version 4, SAS Institute, Cary, NC). In cases where disease incidence data were not normally distributed, analysis of variance and separation of means was performed on arcsin-transformed data, but percent data are reported.

Rating = 0
Plant with no bacterial leaf blight symptoms.

Rating = 1
Plant with a few individual bacterial leaf blight lesions.

Rating = 2
Plant with many individual lesions.

Rating = 3
Plant with individual lesions and small patches of coalesced lesions.

Rating = 4
Plant with medium sized patches of coalesced lesions.

Rating = 5
Plant with large patches of coalesced lesions.

Fig. 1. Bacterial leaf spot severity was evaluated by rating the most severely damaged area on the lettuce plant on a scale of 0 to 5.

In two experiments conducted in 2001, we tested a total of nine products or combinations of products for reduction of disease incidence and severity. Some of the products were not tested further since they did not show promise in these two experiments. Of the six field experiments, those conducted in the fall had significantly greater disease incidence and severity ($P < 0.0001$) than the spring experiments (Tables 2 and 3). Disease incidence was 100% for all treatments planted in the fall of 2002 and 2003. By contrast the average disease incidence across all treatments was 69% in the spring trials. Likewise, the average severity was greater (3.8) in the fall than in the spring (2.2).

Table 2. Efficacy of chemical and biological materials on the incidence of bacterial leaf spot of lettuce (cultivar Vista Verde) in field experiments.

Treatment	Disease incidence					
	Spring 2001		Spring 2002		Fall 2002	Fall 2003
	Spence[w]	Field C[x]	Spence	Field C	Field C	Field C
Control	90 a[y]	47 A	100 A	100 a	100	100
Serenade + Cuprofix	59 b	19 B	100 A	83 b	100	100
Serenade	79 ab	(35)	90 B	93 ab	100	100
Cuprofix	69 ab	31 AB	96 AB	90 AB	100	100
Maneb	78 ab	41 AB	94 AB	90 ab	100	100
Maneb + Cuprofix	66 AB	37 AB	99 AB	78 b	100	100
Actigard	64 ab	25 AB	nt[z]	nt	nt	nt
Copper	76 ab	30 AB	nt	nt	nt	nt
Messenger	74 ab	43 AB	nt	nt	nt	100
Quadris	86 ab	43 AB	nt	nt	nt	nt
P value	0.023	0.014	0.048	0.046		

[w] In this experiment arcsin data were used to conduct ANOVA and separation of means but percent data are reported.

[x] The Serenade treatment had variances that were different from the rest of the treatments in this experiment; therefore, these data could not be analyzed with the experiment. The mean disease incidence is given in parentheses.

[y] Means followed by the same letters are not significantly different at $P \leq 0.05$ according to Tukey-Kramer HSD.

[z] nt = not tested.

Table 3. Efficacy of chemical and biological materials on the disease severity of bacterial leaf spot of lettuce (cultivar Vista Verde) in field experiments.

Treatment	Disease severity[x]					
	Spring 2001		Spring 2002		Fall 2002	Fall 2003
	Spence	Field C	Spence	Field C	Field C[w]	Field C
Control	2.35	2.27	1.94	2.72	3.50 A[y]	4.84 A
Serenade + Cuprofix	2.24	2.28	1.83	2.09	2.23 B	4.38 ABC
Serenade	2.24	2.77	1.98	2.82	3.13 AB	4.81 A
Cuprofix	2.23	2.51	1.69	2.22	2.75 AB	4.30 BC
Maneb	2.35	2.38	2.28	2.24	3.23 AB	4.65 ABC
Maneb + Cuprofix	2.32	2.40	2.02	1.97	2.83 AB	4.25 C
Actigard	2.28	2.27	nt[z]	nt	nt	nt
Copper	2.37	2.41	nt	nt	nt	nt
Messenger	2.23	2.39	nt	nt	nt	4.77 AB
Quadris	2.54	2.39	nt	nt	nt	nt
P value	0.323	0.285	0.611	0.053	0.035	0.002

[x] Disease severity was rated as follows: plants with no visible symptoms = 0, a few individual lesions = 1, many individual lesions = 2, small patches of coalesced lesions = 3, medium sized patches of coalesced lesions = 4 and large patches of coalesced lesions = 5 on the severity scale (Fig. 1).

[y] Means followed by the same letters are not significantly different at $P \leq 0.05$ according to Tukey-Kramer HSD.

[z] nt = not tested

In the two fall experiments, every plant in the field became diseased; therefore, no differences in disease incidence were detected (Fall 2002 and 2003) (Table 2). Disease severity was reduced significantly by a mixture of Serenade and Cuprofix and a mixture of Maneb and Cuprofix in trials conducted in the fall of 2002 and 2003, respectively. In Fall 2002, treatment with Serenade and Cuprofix reduced severity by approximately 36% while in Fall 2003 treatment with Maneb and Cuprofix reduced disease by approximately 10%. These two experiments were the only experiments in which severity was significantly less in some treated plants compared to the controls. Although naturally infested fields rarely reach 100% disease incidence, the data may indicate that biological and chemical treatment reduce severity only under high disease pressure.

The mixture of Serenade and Cuprofix resulted in significantly lower incidence of disease ($P < 0.048$) (Table 2) in three of the four experiments in which disease incidence was less than 100%. For example, at the Spence field site in 2001, treatment with the mixture of Serenade and Cuprofix resulted in a 31% reduction in incidence over the untreated control (Table 2). In one of the three experiments in which the Serenade and Cuprofix mixture reduced disease incidence, treatment with the Maneb and Cuprofix mixture also reduced disease incidence significantly (Spring 2002, Field C) (Table 2). Thus, the mixture of Serenade and Cuprofix reduced either disease incidence or disease severity in four out of six experiments compared to Maneb and Cuprofix which reduced either severity or incidence in only two out of six experiments. In general the individual components of these treatments gave intermediate control that did not differ from either the control or the mixture. Thus, although disease control for the mixture did not differ from either product individually, only the mixture significantly reduced disease. There was one exception to this finding. In a fourth experiment (Spring 2002, Spence) treatment with the mixture Serenade and Cuprofix did not reduce disease, but treatment with Serenade reduced disease by 7%. In all other experiments, treatment with Serenade as a stand-alone product did not reduce disease incidence or disease severity. It is not clear what factors contribute to variability in control using these chemically and biologically based materials.

Copper compounds have a history of being effective in reducing bacterial diseases including bacterial leaf spot caused by *X. campestris* pv. *vitians* (7). Our data are somewhat surprising in that Cuprofix never significantly reduced disease incidence when applied as a stand-alone treatment. Additionally, levels of copper below phytotoxic levels were not effective in control of this disease in other studies (14). In the experiments reported here, phytotoxicity was not evident in any of the plots that received applications containing copper (*data not shown*).

In these experiments, a mixture of Serenade and Cuprofix, a copper based compound, reduced either disease incidence or disease severity in two thirds of the experiments. It would be interesting to evaluate this mixture for control of bacterial leaf spot in organic production systems since Serenade has been approved for use in organic production (1). Although Cuprofix has not been approved for use in organic production to-date, one copper sulfate product has been approved for restricted use in these systems. Moreover, Serenade and copper compounds both reduced Septoria leaf spot on tomato grown under organic management (18). Therefore, the mixture of Serenade and Cuprofix or another copper compound has a potential for use in prevention of bacterial leaf spot in organic production systems. Experiments evaluating the efficacy of this mixture on bacterial leaf spot of lettuce specifically in organic systems are needed.

One drawback to using copper compounds in any production system is the potential build up of resistance to copper in the pathogen population (8). For example, copper resistant strains of *X. campestris* pv. *vesicatoria* have been recovered from tomato samples in California (8). Few reports have evaluated resistance to copper among *X. campestris* pv. *vitians*. In one report that did, none of the five strains isolated from lettuce with bacterial leaf spot in Ohio were resistant to copper (13).

Summary

Since 2001, we have conducted six field trials evaluating chemical and biological treatments for control of bacterial leaf spot of lettuce. In four of the experiments, treatment with the mixture of Serenade and Cuprofix significantly reduced either disease incidence or disease severity regardless of the growing season. The mixture of Maneb and Cuprofix reduced either severity or incidence in two of six experiments. In general the individual components of these treatments gave intermediate control that did not differ from either the control or the mixture; however, in one experiment Serenade reduced disease by 7%. It is not clear if a reduction in disease would increase yield and quality since yield based on market standards did not differ among treatments in experiments in which it was evaluated (*data not shown*).

Although bacterial leaf spot incidence has been high in coastal California during Fall 2004 and Spring 2005, the disease still occurs only sporadically and is dependant on environmental conditions. Preventative application with a mixture of Serenade and Cuprofix may reduce either incidence or severity of bacterial leaf spot but not consistently and the factors influencing variability are unknown. Additionally, the sporadic nature of the disease may prevent prophylactic applications from being economically feasible as an individual strategy. Application of chemicals and biopesticides as preventative treatments may serve as part of an integrated management strategy that includes clean planting material, adequate time between replanting into previously diseased fields, and deployment of host resistance. The data presented here does not indicate that the mixture of Serenade and Cuprofix should be recommended as a stand-alone treatment.

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