



Brief Report

# Efficacy of Postiva™ for Management of Bacterial Diseases of Ornamental Crops

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**Abstract:** Pathogen management commonly includes environmental regulation and sanitization. There are limited numbers of effective registered bactericides. In 3 years of greenhouse trials, Postiva™, a premix of pydiflumetofen (6.9%) and difenoconazole (11.5%), was tested for activity against xanthomonas leafspot of geranium, zinnia, ficus and bacterial wilt of geranium caused by *Ralstonia solanacearum*. Postiva™ applied at 0.73–1.5 L/ha significantly reduced disease incidence and/or severity on each crop tested. Postiva™ applications were similar ( $p = 0.05$ ) to commercially available standards on geranium, zinnia and ficus. Postiva™ (0.73 L/ha) reduced incidence and severity of bacterial wilt similar to that observed with applications of Cease® (9.35 L/ha). Postiva™ may be beneficial in an integrated disease management program to control bacterial diseases. Postiva™ is highly promising as a rotation option to reduce the buildup of bacterial populations resistant to copper compounds and antibiotics that are frequently used in the industry.

**Keywords:** xanthomonas campestris; ralstonia solanacearum; geranium; zinnia



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## 1. Introduction

The production of ornamental crops in the U.S. is an estimated USD 4.4 billion dollar industry that encompasses a diverse assortment of bedding plants, potted flowers, foliage plants, and nursery crops [1]. Ornamental plants have high retail value and are widely planted to improve the aesthetics and value of landscapes and gardens. Plants exhibiting symptoms of disease are significantly less valuable and may be rejected by wholesalers. Each segment of the ornamental industry faces disease challenges from bacterial pathogens [2]. Bacteria cause significant economic loss, causing disease during all phases of production and reducing the quality of finished plants [3,4]. The ornamental industry is complex and plants or cuttings are often started in one production location and shipped to other production facilities for transplanting and finishing [5]. Bacteria that are present on plant material during propagation, may be spread during handling or worker activities [3]. Contaminated water in a greenhouse can contain bacterial pathogens that are disseminated during irrigation [6]. Flood floor and ebb-and-flow production systems that use recirculating irrigation water have been widely adopted in greenhouses to maximize production area and decrease labor costs [7,8]. A significant drawback of producing crops using recycled irrigation water is the dissemination of plant pathogens [3,9]. Frequent irrigation also creates an environment which is favorable for bacterial infection [10]. Once introduced into a facility, bacteria are difficult to eradicate and may survive on greenhouse surfaces or tools [3,11]. These factors make bacterial diseases difficult to control during production and multiple control strategies are often necessary [2,3].

Management of bacterial diseases often includes strict sanitation and bactericide applications to prevent outbreaks and limit spread [3]. Sanitation is very important in bacterial

management, especially to prevent movement of new strains into production facilities [12]. In addition to sanitation, bactericides have proven useful to limit disease [3,4,13]. Many bactericides are formulated with copper, zinc, or other metals [3,4]. Copper products applied alone (copper hydroxide, copper oxychloride, etc.) or in combination with or without mancozeb can provide control of *Xanthomonas* when populations are sensitive to the copper compounds [14]. Excellent control of basil blight caused by *Pseudomonas cichorii* was achieved with sprays of copper-maneb or streptomycin sulfate [13]. Under high disease pressure, streptomycin did not control *X. perforans* on tomato, however, other fungicide and bactericide treatments provided significant levels of control [15]. Bacterial spot of pepper was effectively controlled with copper hydroxide and streptomycin, but not microbial fertilizers or *Bacillus subtilis* [14]. On zonal geranium and poinsettia, titanium dioxide sprays applied preventively effectively controlled *X. hortorum* and *X. axonopodis*, respectively; however, heavy residue made plants unmarketable [4]. Deberdt, Davezies [16] found that a drench of leaf oil from *Pimenta racemosa* provided control of bacterial wilt of tomato in the greenhouse at a rate of 0.14% (v/v). Field trials need to be conducted to verify if sustained levels of control can be achieved with this natural compound. Flaherty, Harbaugh [17] found that daily treatment of geranium plants with *Xanthomonas*-specific bacteriophages resulted in lower disease incidence and severity than plants treated less frequently with bacteriophages or copper sulfate pentahydrate. In in vitro studies, fungicides and antibiotics were tested against bacterial species isolated from ornamental crops and Dithane<sup>®</sup> M45 (mancozeb) was found to effectively limit growth of *Xanthomonas* and *Corynebacterium*, but not *Erwinia* and *Pseudomonas* [18]. Acibenzolar-S-methyl provided good control of *Xanthomonas perforans* on greenhouse-grown tomato seedlings, but did not increase yields once they were planted in the field [15]. However, this compound is not labeled for use on greenhouse ornamentals.

The objective of this research was to determine the effectiveness of applications of Postiva<sup>™</sup> (pydiflumetofen 6.9%, difenoconazole 11.5%) for control of common bacterial pathogens that cause extensive losses during production and have regulatory significance in plant movement. We chose to look at xanthomonas leaf spot of geranium (*X. hortorum* pv. *pelargonii*), zinnia (*X. campestris* pv. *zinnia*), ficus (*X. campestris* pv. *fici*), and bacterial wilt of geranium caused by *Ralstonia solanaceum*.

## 2. Materials and Methods

Trials were conducted at the University of Florida, Mid-Florida Research and Education Center in Apopka, FL and the Syngenta Vero Beach Research Center in Vero Beach, FL, USA.

### 2.1. Experimental Design University of Florida

Bacterial strains of *X. hortorum* pv. *pelargonii* (strain X575), *X. campestris* pv. *fici* (strain 1960) and *R. solanaceum* (strain P673, Phylotype IIB, sequevar 4NPB [19]) were grown for 48 h at  $28 \pm 1$  °C on nutrient agar (NA, Difco Laboratories, Detroit, MI, USA), amended with 0.5% sucrose. After 48 h, bacterial cells were harvested from NA plates, suspended in saline (NaCl, 8.5 g/L) and adjusted spectrophotometrically at  $A_{600}$  to  $1 \times 10^5$ ,  $1 \times 10^8$ ,  $1 \times 10^6$  colony-forming units (CFU) per ml, for respective strains. Inoculum was used within 30 min of preparation.

Geranium ('American Bright Red') and *Ficus microcarpa* cuttings were rooted and planted into 9 cm and 12.7 cm pots containing Jolly Gardener Potting Mix #2. Plants were fertilized with 1.5 g/pot Osmocote Plus (15N-9P-12K with micronutrients) and were hand watered three times a week. Plants were allowed to establish and grow to approximately 15 cm in high. The greenhouse temperatures were maintained between 19–32 °C. Leaves and stems of geraniums and ficus were sprayed until run-off using handpump sprayers with bacterial suspensions of respective *Xanthomonas* species and enclosed in clear polyethylene bags for 24 h. To mimic the high humidity and rainfall that is experienced during cultivation of *F. macrocarpa* plants in Florida and many tropical regions of the world, plants were unbagged then misted every 10 min during daylight hours for 5 s. *Xanthomonas* leaf

spots for geranium and ficus were counted 2 weeks after inoculation. The bacterial wilt suspension of *R. solanacearum* (20 mL) was poured around the base of each plant. Care was taken to avoid contact with the stem. Plants were evaluated for wilt symptoms over a 5-week period for percent of wilt symptoms. At the completion of all tests, random re-isolations were done to confirm pathogen identity on either NA or tetrazolium chloride medium [20]. Commonly used bactericides were added as treatments (Table 1) along with a noninoculated treatment. Each treatment consisted of 10 plants in a randomized block design. The experiment was repeated 3 times for the xanthomonas leaf spot of geranium. To confirm further activity, the test was also conducted once for both the leaf spot of ficus and bacterial wilt of geranium. Data within each test were analyzed by analysis of variance (ANOVA) and Tukey's LSD test ( $p = 0.05$ ) using Sigma Plot 13 (Systat Software Inc., San Jose, CA, USA).

**Table 1.** Bactericide treatments used in this study.

Product Name	Active Ingredient	Manufacturer	Rate
Postiva™	Pydiflumetofen (6.9%) + Difenconazole (11.5%)	Syngenta Crop Protection LLC	0.73, 1.02, 1.5 L/ha
CuPRO® 5000	Copper Hydroxide (61.3%)	SePRO Co	1.75 kg/ha
Cease®	<i>Bacillus subtilis</i> , QST 713 strain (1.34%)	AgraQuest	9.35 L/ha
DaconilZN®	Chlorothalonil (38.5%)	Syngenta Crop Protection LLC	2.34 L/ha
A19649B	Pydiflumetofen (6.9%)	Syngenta Crop Protection LLC	1.02/ha

## 2.2. Experimental Design Syngenta Crop Protection

For inoculum production, a strain of *X. campestris* pv. *zinniae* (strain 170-X) was cultured on NA plates for 3 days at 22–24 °C. Bacteria were harvested by flooding the media surface with sterile water and gently rubbing with a rubber spatula. Suspensions were adjusted using a spectrophotometer at  $A_{580}$  and adjusted to between  $1 \times 10^5$  to  $1 \times 10^6$  CFU/per mL. Inoculum was used within 30 min of preparation. Zinnia (*Zinnia elegans*) 'Benary Giant Pink' were grown from seed and transplanted into 12 cm pots containing Promix BX mix (Premier Tech Home & Garden., Rivière-du-Loup, QC, Canada). All plants were fertilized with 1 g Osmocote (15N-9P-12K) and were grown in a greenhouse maintained at 23 °C for 4 weeks until plants were approx. 15 cm high. Plants were sprayed to runoff with the bacterial suspension and enclosed in polyethylene bags for 2 days under 50% shade and then returned to the greenhouse bench. Symptom development was monitored for 3–4 weeks. Disease severity was rated as percent of damaged leaf area, and plant health was rated using a scale developed to represent commercial standards for ornamentals where 1 = dead plant and 9 = 100% healthy plant. To confirm pathogenicity *Xanthomonas* was reisolated onto NA plates. Products were applied at 276 kpa using a backpack sprayer and a single nozzle spray boom with flat fan nozzles (TeeJet XR-8002, Spraying Systems Co., Wheaton, IL, USA) directed at a 45° angle to the plants, and at a height of 20–30 cm above the plants. The plants were treated 24–48 h prior to inoculation. There were 6–8 plant replicates per treatment in a randomized block design with industry standards included (Table 1) and a noninoculated control. The zinnia trial was conducted twice. Data were analyzed using JMP Pro 14 (SAS Institute, Cary, NC, USA). Treatment means were analyzed with analysis of variance (ANOVA) and treatment means were separated using Fisher's least significant difference (LSD) test ( $p = 0.05$ ).

## 3. Results

### 3.1. Efficacy on *Xanthomonas* Leaf Spot of Geranium

Postiva™ was found to be very effective in reducing the number of xanthomonas leafspots on geranium (Table 2). Three rates of Postiva™ were tested in this study with

one to three commercial standards (Table 1). All three rates of Postiva™ in each of the three trials on geranium significantly reduced leaf spot numbers when compared to the inoculated control (Table 2). There was no clear trend between effectiveness of the product and application rate. Disease was present but less severe with the Postiva™ treatments, with between 26.4 and 74.5% reduction in leafspots per plant for all three rates tested. Cease®, the commercial standard, also had leafspots present with an average reduction of 51.2% in leafspots per plant. When tested, the copper- and Zn-containing products performed well in reducing the number of leaf spots (Table 2). No treatment completely controlled the disease.

**Table 2.** Xanthomonas leaf spot counts on ‘American Bright Red’ geranium plants and mean xanthomonas blighted leaves on *Ficus macrocarpa*.

Treatments (Rate/ha)	Geranium						<i>Ficus macrocarpa</i>	
	2019		2020				2021	
	Test 1		Test 2		Test 3			
	Mean Leafspots <sup>x</sup>	LSD <sup>y</sup>	Mean Leafspots	LSD	Mean Leafspots	LSD	Mean Blighted Leaves	LSD
Negative control	0	a	0	a	0	a	0	a
Inoculated control	171.1	d	183	e	77.8	e	15.2	d
Postiva™ (0.73 L/ha)	77.5	bc	47.6	bcd	48	cd	11.2	bc
Postiva™ (1.02 L/ha)	126	cd	81	d	30	bc	11.1	bc
Postiva™ (1.5 L/ha)	101.3	bc	46.5	bcd	26.5	abc	8.9	ab
Cease® (9.35 L/ha)	56.6	ab	60.1	cd	62.6	de	-	-
CuPRO® 5000 (1.75 kg/ha)	- <sup>z</sup>	-	15.9	ab	2.2	a	-	-
DaconilZN® (2.34 L/ha)	-	-	27.7	abc	8.9	ab	-	-

<sup>x</sup> Mean leafspot count for each treatment. <sup>y</sup> Column means with a letter in common are not significantly different according to Fisher’s least significant difference (LSD) test ( $p = 0.05$ ). <sup>z</sup> Products not tested.

### 3.2. Efficacy on Xanthomonas Leaf Spot of Ficus

A lower level of control of xanthomonas leaf spot was measured on *F. macrocarpa* than geranium. The growing conditions with the frequent misting presented a much harder control scenario favoring disease development. *Ficus macrocarpa* is marketed as an ornamental plant that is used as an indoor bonsai, as a hedge plant, or as a large banyan in landscape plantings. Xanthomonas leaf spot of ficus is very hard to control in subtropical to tropical regions of the world with high rainfall and humidity. When leaf spots develop on *F. macrocarpa* the plant responds by defoliating all infected leaves, frequently leaving nothing but bare stems. Postiva™ did significantly lower the number of infected leaves (Table 2). More testing in landscape environments will need to be done to prove effectiveness.

### 3.3. Bacterial Wilt Efficacy

In the *Ralstonia* trial, wilt severity ranged from 0 to 24% at the second rating for all treatments (Table 3). Disease progress decreased at the low and high rate of Postiva™ and for the Cease® treatment. By the final rating, there was >60% wilt in the control treatment, and <20% wilt at the low rate of Postiva™. The high and mid-rate of Postiva™ and Cease® had moderate levels of disease (42–59%) (Table 3). More research is needed to determine an effective rate dosage effect. There is little tolerance for bacterial wilt in the industry. Exclusion would still be the best choice in managing this disease. In a previous study, *Ralstonia* infection of geranium roots was prevented with phosphorus acid drenches, however, no protection was observed from wound inoculation (Norman, Chen [21]).

**Table 3.** *Ralstonia* bacterial wilt incidence on ‘American Bright Red’ geranium plants.

Treatment (Rate/ha)	<i>Ralstonia</i> /Geranium 2019			
	14 DAI <sup>y</sup>		28 DAI	
	Mean % Wilted Foliage	LSD <sup>z</sup>	Mean % Wilted Foliage	LSD
Uninoculated control	0	a	0	a
Inoculated control	23	b	62.5	c
Postiva <sup>TM</sup> (0.73 L/ha)	3.5	a	18	ab
Postiva <sup>TM</sup> (1.02 L/ha)	24	b	54.5	c
Postiva <sup>TM</sup> (1.5 L/ha)	20.5	b	59	c
Cease <sup>®</sup> (9.35 L/ha)	12.5	ab	42	bc

<sup>y</sup> Days After Inoculation. <sup>z</sup> Column means with a letter in common are not significantly different according to Fisher’s least significant difference (LSD) test ( $p = 0.05$ ).

### 3.4. Efficacy on *Xanthomonas* Leaf Spot of Zinnia

All rates of Postiva<sup>TM</sup> significantly lowered the percent of xanthomonas damage on zinnia foliage ( $p < 0.05$ ). Results were similar for both years of testing. Again, there was no clear distinction in application rate and disease severity. Similar level of control was also achieved with the commercial standards Cease<sup>®</sup>, DaconilZN<sup>®</sup>, and CuPRO 5000<sup>®</sup> (Table 4). Plant health was significantly greater for all Postiva<sup>TM</sup> treatments and the standards then for the inoculated control (Table 4).

**Table 4.** *Xanthomonas* leaf spot severity on zinnia ‘Benary Giant Pink’ evaluated in 2019 and 2020.

Treatment (Rate/ha)	2019				2020			
	Mean % Leaf Damage <sup>w</sup>	LSD <sup>y</sup>	Plant Health <sup>x</sup>	LSD	Mean % Leaf Damage	LSD	Plant Health	LSD
Uninoculated control	1.8	b	8.4	a	0.5	c	8.8	a
Inoculated control	23.6	a	3.8	b	20.5	a	3	d
Postiva <sup>TM</sup> (0.73 L/ha)	2.8	b	7.8	a	4.8	bc	7.3	abc
Postiva <sup>TM</sup> (1.02 L/ha)	3.7	b	7.2	a	10.5	abc	6	c
Postiva <sup>TM</sup> (1.5 L/ha)	0.6	b	8.4	a	12	abc	6	c
CuPRO 5000 <sup>®</sup> (1.75 kg/ha)	4.4	b	6.6	a	- <sup>z</sup>	-	-	-
Cease <sup>®</sup> (9.35 L/ha)	4.1	b	7.4	a	14	ab	6.3	bc
DaconilZN <sup>®</sup> (2.34 L/ha)	1.9	b	8.2	a	-	-	-	-
A19649B (6.9%)(1.02 L/ha)	1.8	b	8.4	a	4.2	bc	8.3	ab

<sup>w</sup> Mean leafspot damage (%) for each treatment. <sup>x</sup> Plant health scale developed to represent commercial standards for ornamentals where 1 = dead plant and 9 = 100% healthy plant. <sup>y</sup> Column means with a letter in common are not significantly different according to Fisher’s least significant difference (LSD) test ( $p = 0.05$ ). <sup>z</sup> Products not tested.

## 4. Discussion

The Fungicide Resistance Action Committee (FRAC) places fungicides into MOA groups based on mode of action. Postiva<sup>TM</sup> is a combination of pydiflumetofen (6.9%) and difenoconazole (11.5%) in MOA groups 7 and 3, respectively. Fungicides within MOA 7 are succinate-dehydrogenase inhibitors (SDHI). They work by blocking succinate dehydrogenase, an enzyme involved in fungal cell respiration. Difenoconazole is a member of MOA group 3, the demethylation inhibitors (DMI-fungicides). DMIs inhibit fungal growth by inhibiting the biosynthesis of ergosterol which is a major component of the plasma membrane of certain fungi. It is not clear which component in Postiva<sup>TM</sup> provided protection from bacterial diseases in these trials, however the experimental pydiflumetofen treatment in a single trial on zinnia suggested that control may be coming from the pydiflumetofen component. Synergism between the chemistries may also be present if the compounds have different modes of action on the bacterial cell [22]. Other possibilities include the activation of a systemic activation response (SAR) within the host plant. Regardless of the mechanism, additional research is needed to determine how it is limiting bacterial disease expression.

Similar results were observed in one study in Tennessee, where Postiva™ foliar sprays provided control of pseudomonas leaf spot on magnolia at 0.73–1.5 L/ha [23]. It is clear from this research and others that Postiva™ would be beneficial in an integrated disease management program to control bacterial diseases. Postiva™ is highly promising as a rotation option with other bactericides. It would also help reduce the buildup of bacterial populations resistant to copper compounds and antibiotics that are frequently used in the industry. Additional evaluations will be necessary to determine if Postiva™ is effective against *Erwinia*, *Agrobacterium*, and other bacterial species.

## 5. Conclusions

Our research illustrates that the fungicide Postiva™ is also bactericidal and effective in controlling bacterial disease in ornamental plant production. There are few bactericides that are effective in controlling bacterial diseases in crop production. Traditional products frequently contain metals or antibiotics which can be harmful to the environment or end consumer. These findings may be of benefit to a wide range of ornamental and agronomic crops that suffer from bacterial pathogens.

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