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Sensibility of *Xanthomonas vasicola* pv. *vasculorum* to chemicals and efficiency of the chemical control of bacterial leaf streak on corn plants

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ABSTRACT. Bacterial leaf streak caused by *Xanthomonas vasicola* pv. *vasculorum* is an emerging disease for corn production around the world. However, information on management of this disease is still limited. This study aimed to determine the *in vitro* sensibility of X. vasicola pv. vasculorum to different chemicals and to evaluate the control of bacterial leaf streak on corn under greenhouse conditions. In vitro tests were carried out with kasugamycin, copper sulfate, copper oxychloride, copper hydroxide, cuprous oxide, bioactive copper, mancozeb, chlorothalonil, methyl thiophanate, and tebuconazole at the dosages of 1, 5, 10, 20, 50, 100, 200, and 400 µg mL⁻¹. Four strains of *X. vasicola* pv. *vasculorum* were included in the study. The minimal inhibitory concentration for kasugamycin ranged from 50 to 200 µg mL⁻¹, whereas to the inorganic copper compounds varied from 5 to 50 μ g mL⁻¹ and to the bioactive copper was 100 μ g mL⁻¹. Further, mancozeb and tebuconazole inhibited the bacterial growth at the dosage ranging from 5 to 20 µg mL⁻¹ and 50 to 400 µg mL⁻¹, respectively, depending on the X. vasicola pv. vasculorum strain. Chlorothalonil and methyl thiophanate did not inhibit the growth of the bacterium at any tested concentration. The control of bacterial leaf streak under greenhouse conditions was investigated on corn plants of the cultivar IPR 164 at the V3 phenological vegetative stage, sprayed with kasugamycin (3 mL L⁻¹), copper oxychloride (1.5 mL L^{-1}), bioactive copper (1 mL L^{-1}), mancozeb (2 g L^{-1}), tebuconazole (1 mL L^{-1}), and chlorothalonil (2 mL L^{-1}). The corn plants were inoculated with a 10^8 CFU mL⁻¹ suspension of the RL1 strain of X. vasicola pv. vasculorum. Only copper oxychloride significantly reduced disease severity. However, this copper compound caused phytotoxicity to the corn plants at the tested concentration.

Keywords: gram-negative bacteria; mancozeb; copper compound; chemical control.

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Introduction

Bacterial leaf streak of corn (*Zea mays* L.) was first reported in South Africa in 1949 (Dyer, 1949). This disease is caused by the bacterium *Xanthomonas vasicola* pv. *vasculorum* (Cobb 1894) (Lang et al., 2017) and was recently reported in corn fields in the United States (Damicone, Cevallos, & Olson, 2018; Jamann, Plewa, Mideros, & Bissonnette, 2019; Korus et al., 2017; Lang et al., 2017) and in Argentina (Plazas et al., 2018). In 2018, the disease was also first reported in corn fields in the western region of the state of Paraná, Brazil (Leite Júnior et al., 2018a).

The initial symptoms of the disease include the presence of small translucent and yellowish flecks on leaves, which expand along the interveinal spaces, forming long streaks with wavy and irregular edges (Korus et al., 2017; Lang et al., 2017). The lesions are intense yellow to brown and can coalesce and cover a large area of the leaf blade. The lesions can vary in shape, and size, according to the corn genotype susceptibility and environmental conditions (Leite Júnior et al., 2018b). The initial symptoms of bacterial leaf streak can appear in the early vegetative stages of the corn plants, such as V4 (Broders, 2017) or V7 (Leite Júnior et al., 2018b). As season progresses, the disease can move to the upper leaves of the plant (Broders, 2017). *X. vasicola* pv. *vasculorum* can also infect ear husks (Leite Júnior et al., 2018b).

Bacterial leaf streak of corn has already been observed in different hybrids and cultivars of corn, popcorn, and sweet corn, indicating the susceptibility of many commercially available genotypes (Broders, 2017; Lang et al., 2017). However, susceptibility to the disease may vary considerably among the different genotypes (Leite Júnior et al., 2018b; Ortiz-Castro et al., 2020).

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X. vasicola pv. *vasculorum* is a gram-negative, rod-shaped, mobile, non-fluorescent, and non-fermentative bacterium that produces yellow mucoid colonies in nutrient agar (NA) (Korus et al., 2017; Plazas et al., 2018; Leite Júnior et al., 2018a). However, the bacterium is not considered a quarantine pest in Brazil (MAPA, 2018a and b).

High temperature and humidity favor the development of bacterial leaf streak of corn (Broders, 2017). Considering the other plant diseases caused by *Xanthomonas* spp., the bacterial leaf streak bacterium probably survives in crop debris and alternative hosts and can spread by wind, and rain and irrigation waters (Broders, 2017). Alternative hosts such as weeds and volunteer corn plants may serve as sources of primary inoculum, and bacterial exudates from corn leaf lesions probably serve as secondary inoculum during the growth season (Broders, 2017; Leite Júnior et al., 2018b; Hartman, Tharnish, Harbour, Yuen, & Jackson-Ziems, 2020; Ortiz-Castro et al., 2020). *X. vasicola* pv. *vasculorum* does not require wounds to infect the host plants and probably penetrates the leaf tissue through natural openings such as the stomata (Leite Júnior et al., 2018b). Evidences suggest that *X. vasicola* pv. *vasculorum* can be transmitted through corn seeds (Arias et al., 2020). Arias et al. (2020) further investigated the role of corn seeds in the epidemics of bacterial leaf streak and the potential transmission of the bacterium from seeds to seedlings. They suggested that the levels of *X. vasicola* pv. *vasculorum* in corn seeds from natural contaminations were very low to result in symptomatic seedlings. Seeds from a small number of diseased corn fields produced quantitative polymerase chain reaction (qPCR)-positive seedlings, but they did not develop any symptoms of the disease (Arias et al., 2020).

Because of the implications of an emergent corn disease in important Brazilian corn-producing regions and the lack of information regarding the causal agent and disease management, including chemical control, this study aimed to determine the sensibility to chemicals of *X. vasicola* pv. *vasculorum* strains isolated from commercial corn fields in the state of Paraná, Brazil, and to evaluate the efficiency of the chemical control of bacterial leaf streak in corn plants under greenhouse conditions.

Material and methods

Bacterial strains

Xanthomonas vasicola pv. *vasculorum* strains RL1, RL2, XVV1, and XVV2 were isolated from symptomatic corn leaves collected from commercial corn fields located in the western region of the state of Paraná, Brazil, in the 2018 season (Leite Júnior et al., 2018a). The strains were identified as *X. vasicola* pv. *vasculorum* based on PCR tests by using Xvv3 and Xvv5 primers, as described previously (Lang et al., 2017). Stock cultures of all four strains were preserved at the Collection of Plant Pathogenic Bacteria of the Plant Bacteriology Laboratory of the IDR-Paraná, Londrina, Paraná State, Brazil.

Determination of the sensibility of X. vasicola pv. vasculorum strains to chemicals

The sensibility of *X. vasicola* pv. *vasculorum* strains to inorganic and organic chemicals and to the antibiotic kasugamycin was tested at the dosages of 1, 5, 10, 20, 50, 100, 200, and 400 µg mL⁻¹ of active ingredient (Table 1). Copper-based compounds, i.e., copper sulfate, copper oxychloride, copper hydroxide, and cuprous oxide, were added to the nutrient agar (NA) medium before autoclaving (120°C for 20 min.), and the pH was adjusted to 7.0-7.2. Bioactive copper, kasugamycin, mancozeb, chlorothalonil, methyl thiophanate, and tebuconazole were prepared in sterile distilled water and added to the NA medium after autoclaving.

Table 1. Chemicals tested for the in vitro inhibition of Xanthomonas vasicola pv. vasculorum and for the chemical control of bacterial
leaf streak of corn under greenhouse conditions.

Chemical	Active ingredient in the	Chemical group	Type ²
	commercial product (% w/w) ¹		
Kasugamycin	2.0	Antibiotic	Fungicide/bactericide
Copper sulfate pentahydrate	25.4	Inorganic copper	Contact Fungicide/bactericide
Copper oxychloride	35.0	Inorganic copper	Contact Fungicide/bactericide
Copper hydroxide	35.0	Inorganic copper	Contact Fungicide/bactericide
Cuprous oxide	75.0	Inorganic copper	Contact action fungicide
Bioactive copper	5.0	Inorganic and organic copper	Organomineral leaf fertilizer
Mancozeb	75.0	Alkylenebis (dithiocarbamate)	Contact fungicide/acaricide
Chlorothalonil	72.0	Carbonitrils	Protective fungicide
Methyl thiophanate	50.0	Benzimidazole	Systemic fungicide
Tebuconazole	20.0	Benzimidazole	Systemic fungicide

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Chemical control of corn bacterial leaf streak

The *X. vasicola* pv. *vasculorum* strains were grown in NA plates for 24–72h at 28°C. Bacterial suspensions were adjusted to 10^8 CFU mL⁻¹ in spectrophotometer (DO₆₀₀ = 0.1), and 5 µL aliquots of the bacterial suspension of each strain were spot deposited on the NA plates containing the different concentrations of the chemicals and incubated at 28°C. Bacterial growth was assessed at 24, 48, and 72h after incubation, by observing the presence or absence of bacterial growth. The experimental design was completely randomized with four replications. The experiment was performed twice.

Evaluation of chemicals to control of bacterial leaf streak of corn under greenhouse conditions

The experiment was performed in a semi-climatized greenhouse at the Experimental Station of Londrina of the IDR–Paraná, Londrina, Paraná State, Brazil. Seeds of the corn cultivar IPR 164 were sown in an 8 L pot containing a mixture of soil and sand (1:1). Seven days after seedling emergence, thinning was performed, leaving three plants per pot.

The plants at the V3 phenological vegetative stage, approximately 12 days after sowing, were sprayed with the chemicals until runoff. The chemicals were applied using a 5 L hand sprayer (Brudden Practical; Brudden Equipamentos Ltda, Pompéia, São Paulo State, Brazil). The chemicals and doses of the active ingredient tested were: kasugamycin (3 mL L⁻¹), copper oxychloride (1.5 mL L⁻¹), bioactive copper (1 mL L⁻¹), mancozeb (2 g L⁻¹), tebuconazole (1 mL L⁻¹), and chlorothalonil (2 mL L⁻¹). Control plants were sprayed with water.

As the tested products have preventive/protective action against plant pathogens, the plants were inoculated 24h after the application of the products. Corn plants were spray-inoculated with a 10⁸ CFU mL⁻¹ bacterial suspension of the *X. vasicola* pv. *vasculorum* RL1 strain and maintained in humid chamber for 24h before and after inoculation. The incidence and severity of bacterial leaf streak were assessed at 10 and 15 days after inoculation, respectively, on the third, fourth, and fifth leaves. Disease severity was assessed by estimating the percentage of the area covered by the lesions in each leaf according to the scale proposed by Robaina, Longhi, Zeffa, Gonçalves, and Leite Júnior (2020).

The experimental design was completely randomized with seven treatments and four replications. The experimental unit comprised three plants, and the experiment was performed twice. Incidence data were submitted to Kruskal–Wallis non-parametric analysis at 0.05 probability and to the false discovery rate test. The severity data were subjected to the analysis of variance, and the means were separated by the Tukey test at 0.05 probability. All analyses were performed using the EasyAnova and Agricolae packages from R program (http://www.r-project.org).

Results

Sensibility of X. vasicola pv. vasculorum to chemicals

The strains of *X. vasicola* pv. *vasculorum* had differences in sensibility to kasugamycin, copper-based compounds, and the other chemicals tested (Table 2). The minimal inhibitory concentration (MIC) of kasugamycin for the RL1, RL2, and XVV2 strains was 200 μ g mL⁻¹, whereas for the strain XVV1 was at just 50 μ g mL⁻¹ (Table 2). On the other hand, the growth of all *X. vasicola* pv. *vasculorum* strains was inhibited by at least one copper compound at the MIC of 20 μ g mL⁻¹, except in the case of the bioactive copper (Table 2). However, differences in sensibility to copper compounds were observed among the strains. For instance, the RL1 strain was more sensitive to cuprous oxide with a MIC of 20 μ g mL⁻¹, whereas the growth inhibition of this bacterial strain by copper sulfate, copper oxychloride, and copper hydroxide started only at the concentrations of 50 μ g mL⁻¹ (Table 2). Furthermore, the RL2 strain showed the highest variability in sensibility to copper-based products. The MIC for the cupric compounds of this strain ranged from only 5 μ g mL⁻¹ in the case of copper oxychloride up to 100 μ g mL⁻¹ for the bioactive copper (Table 2). Further, all *X. vasicola* pv. *vasculorum* strains were less sensitive to bioactive copper, with MIC of 100 μ g mL⁻¹ (Table 2).

The *X. vasicola* pv. *vasculorum* strains also had differences in sensibility to mancozeb and tebuconazole. Although the MIC for mancozeb of the XVV1 and RL2 strains were only 5 and 10 μ g mL⁻¹, respectively, it was 20 μ g mL⁻¹ for the RL1 and XVV2 strains (Table 2). The MIC of tebuconazole for the RL1 strain was 100 μ g mL⁻¹, and 50 μ g mL⁻¹ for the RL2 and XVV2 strains (Table 2). The XVV1 strain was inhibited only at 400 μ g mL⁻¹ of tebuconazole (Table 2). On the other hand, the growth of the *X. vasicola* pv. *vasculorum* strains was not inhibited by chlorothalonil and methyl thiophanate even at the highest tested dosage of 400 μ g mL⁻¹ (Table 2).

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Table 2. Sensitivity in vitro of the RL1, RL2, XVV1, and XVV2 strains of Xanthomonas vasicola pv. vasculorum to different chemicals.

Chemical	Bacterial strain																											
		RL1						RL2							XVV1							XVV2						
	$0^{1} 1 5$	10	20	50	100	200	400	015	10	20	50	100	200	400	015	10	20	50	100	200	400	015	10	20	50	100	200	400
Kasugamycin	+2 ++	+	+	+	+	-	-	+++	+	+	+	+	-	-	+++	+	+	-	-	-	-	+++	+	+	+	+	-	-
Copper sulphate	+ ++	+	+	-	-	-	-	+ + +	+	-	-	-	-	-	+++	-	-	-	-	-	-	+++	+	+	-	-	-	_
Copper oxychloride	+ ++	+	+	-	-	-	-	+ + -	_	-	-	-	-	-	+++	+	+	-	-	-	-	+++	_	-	-	-	-	_
Copper hydroxide	+ ++	+	+	-	-	-	-	+ + +	_	-	-	-	-	-	+++	+	+	-	-	-	-	+++	+	+	-	-	-	_
Cuprous oxide	+ ++	+	_	-	_	-	-	+ + +	+	+	_	-	-	-	+++	+	+	_	-	-	-	+++	+	_	_	-	-	_
Bioactive copper	+ ++	+	+	+	_	-	-	+ + +	+	+	+	-	-	-	+++	+	+	+	-	-	-	+++	+	+	+	-	-	_
Mancozeb	+ ++	+	_	-	_	-	-	+ + +	_	_	_	-	-	-	++-	_	_	_	-	-	-	+++	+	_	_	-	-	_
Chlorothalonil	+ ++	+	+	+	+	+	+	+++	+	+	+	+	+	+	+++	+	+	+	+	+	+	+++	+	+	+	+	+	+
Methyl thiophanate	+ ++	+	+	+	+	+	+	+ + +	+	+	+	+	+	+	+++	+	+	+	+	+	+	+++	+	+	+	+	+	+
Tebuconazole	+ ++	+	+	+	-	-	-	+++	+	+	_	-	_	-	+++	+	+	+	+	+	-	+++	+	+	_	-	-	-

¹Concentration of the chemical compound (µg mL⁻¹). For the copper compounds, the concentration is on metallic copper (Cu⁺²). ²+, presence of bacterial growth; –, absence of bacterial growth.

Effect of chemicals on the control of bacterial leaf streak of corn under greenhouse conditions

Typical symptoms of bacterial leaf streak showed up as small, water-soaked, and translucent flecks on the leaves, approximately 7 days after inoculation. These initial symptoms progressed to larger yellow to brown lesions with wavy margins and were restricted to the interveinal spaces. The symptoms were similar to those observed in corn plants under field conditions, as previously reported for bacterial leaf streak (Broders, 2017; Damicone et al., 2018; Leite Júnior et al., 2018b).

The corn plants that received sprays of copper oxychloride had the lowest incidence of bacterial leaf streak on the three leaves evaluated (Table 3). The other tested products did not significantly reduce the incidence of bacterial leaf streak in the corn plants at the concentrations tested (Table 3). However, copper oxychloride at 1.5 mL L⁻¹ was highly phytotoxic to the corn plants. Further, bioactive copper was also phytotoxic to the corn plants, but to a less extent compared to copper oxychloride.

Treatment		Incide	$(\%)^{1,2,3}$		Severity (%) ^{1,2,4}							
		Leaf		Mean		Leaf		Mean				
	3 rd	4^{th}	5^{th}	•	3 rd	4^{th}	5 th	-				
Control (water)	91.8 ab	100.0 a	100.0 a	97.3 a	23.38 ab	38.29 ab	28.33 a	30.00 a				
Copper oxychloride	50.0 b	66.7 b	50.0 b	55.6 b	6.25 b	11.25 b	6.67 b	8.06 b				
Bioactive copper	100.0 a	100.0 a	83.3 a	94.4 ab	17.33 ab	26.08 ab	16.88 ab	20.10 ał				
Mancozeb	100.0 a	100.0 a	100.0 a	100.0 a	33.46 a	44.04 a	25.71 ab	34.40 a				
Tebuconazole	100.0 a	100.0 a	100.0 a	100.0 a	23.25 ab	23.18 ab	25.04 ab	23.82 al				
Chlorothalonil	100.0 a	100.0 a	100.0 a	100.0 a	26.04 ab	31.05 ab	15.88 ab	24.33 al				
Kasugamycin	83.3 ab	91.8 a	83.5 a	86.2 ab	35.83 a	51.00 a	31.88 a	39.57 a				
CV (%)	-	-	-	-	33.74	27.86	27.25	25.00				

 Table 3. Incidence and severity of bacterial leaf streak on corn plants treated with chemical compounds and inoculated with the RL1 strain of Xanthomonas vasicola pv. vasculorum under greenhouse conditions.

¹Incidence and severity of bacterial leaf streak were assessed at 10 and 15 days after inoculation, respectively, on the third, fourth and fifth leaves. ²Original means; data were transformed by \sqrt{x} + 0.5 for statistical analyses. ³Values followed by the same letter in the column are not significantly different from each other according to the false discovery rate test. ⁴Values followed by the same letter in the column are not significantly different from each other according to the false discovery rate test. ⁴Values followed by the same letter in the column are not significantly different from each other according to the false discovery rate test.

Regarding the severity of bacterial leaf streak, copper oxychloride was also the most effective chemical, reducing significantly the diseased area compared to mancozeb, kasugamycin and the control (Table 3). However, bioactive copper, tebuconazole, and chlorothalonil did not significantly reduce the severity of bacterial leaf streak compared to the control (Table 3).

Discussion

The sensibility of *X. vasicola* pv. *vasculorum* to chemical products and the chemical control of bacterial leaf streak of corn has not yet been reported (Ortiz-Castro et al., 2020). Therefore, to our knowledge, this is the first study regarding sensibility of *X. vasicola* pv. *vasculorum* to chemical and the control of the bacterial disease of corn. Our results revealed that the sensibility of *X. vasicola* pv. *vasculorum* strains to kasugamycin, copper-based chemicals, and some fungicides differed slightly. Furthermore, a few chemicals were effective in controlling bacterial leaf streak of corn under greenhouse conditions, i.e. copper oxychloride.

Chemical control of corn bacterial leaf streak

Mancozeb was the most effective chemical to inhibit the growth of all four strains of *X. vasicola* pv. *vasculorum* included in this study, with MIC ranging from 5 up to 20 μ g mL⁻¹ (Table 2). This result is in agreement with studies on the bactericidal activity of mancozeb against plant pathogenic bacteria, such as those reported for *Xanthomonas citri* pv. *citri*, the causal agent of citrus canker (Meneguim et al., 2007) and *Pantoea ananatis*, which causes the bacterial white spot disease on corn (Bomfeti et al., 2007). Bomfeti et al. (2007) also found that among several chemicals tested, including antibiotics and copper-based compounds, only mancozeb at the tested concentrations of 95, 190, and 285 μ g mL⁻¹ completely inhibited *P. ananatis* growth *in vitro*. In addition, the foliar application of mancozeb resulted in an efficient control of bacterial white spot in corn plants naturally infected with *P. ananatis* (Bomfeti et al., 2007).

Copper-based compounds also inhibited the growth of *X. vasicola* pv. *vasculorum* (Table 2). Meneguim et al. (2007) reported the *in vitro* MIC of 100 μ g mL⁻¹ of copper sulphate for *Xanthomonas citri* subsp. *citri*. Our study showed that the growth of *X. vasicola* pv. *vasculorum* was inhibited *in vitro* by copper sulfate at lower concentration, 50 μ g mL⁻¹ of metallic copper (Table 2). Therefore, copper-based compounds have the potential for the control of bacterial leaf streak on corn.

Copper compounds are widely used in the control of several bacterial plant diseases (Lamichhane et al., 2018; Leite Júnior, 2000), but their efficiency in disease control has been shown to vary (Lamichhane et al., 2018). The presence of bacterial populations resistant to copper may explain such variable results (Behlau, Gochez, & Jones, 2020; Marco & Stall, 1983; Cooksey, 1990).

Behlau, Belasque Júnior, Bergamin Filho, and Leite Júnior (2007) confirmed that the incidence and severity of citrus canker was reduced by approximately 44% and 37%, respectively, in 'Pera' sweet orange [*Citrus sinensis* (L.) Osbeck] trees treated with copper oxychloride compared to those non-treated. Recent studies have revealed that treatments with insoluble and soluble copper formulations significantly reduce the incidence of citrus canker on leaves of sweet orange trees (Behlau, Scandelai, Silva Júnior, & Lanza, 2017). Although copper-based bactericides may be effective for the control of bacterial diseases, they do not completely eliminate the bacterial inoculum, and frequent applications in citrus are necessary to prevent losses (Behlau et al., 2020). The continuous application of copper may trigger the selection of resistant strains and favor a gradual increase in the frequency of resistance in the bacterial population, reducing the effectiveness of these chemicals for bacterial diseases control (Sundin, Jones, & Fulbright, 1989). However, corn is an annual crop; hence, many applications may not be necessary to control diseases, and copper compounds are not regularly used in this crop. As observed in our study, phytotoxicity may be a problem in corn plants sprayed with copper compounds such as copper oxychloride and copper hydroxide (Bomfeti et al., 2007); thus, this is a major concern when considering the use of copper-based products for foliar sprays in corn.

The results of this study regarding the chemical control of bacterial leaf streak under greenhouse conditions showed some differences regarding the sensitivity of X. vasicola pv. vasculorum to the chemicals shown in the *in vitro* tests. Despite copper-based compounds have not showed effectiveness in controlling bacterial diseases of corn, such as Goss bacterial wilt caused by Clavibacter michiganensis subsp. Nebraskensis (Mehl, Weems, Ames, & Bradley, 2015; Oser, Jackson-Ziems, & Brungardt, 2013), sprays of copper oxychloride significantly reduced the incidence and severity of bacterial leaf streak on the corn plants. On the other hand, kasugamycin and mancozeb, which inhibited X. vasicola pv. vasculorum growth in vitro, did not reduce the disease under greenhouse conditions. The experimental conditions may have influenced the effectiveness of these chemicals to control the bacterial disease (Hartman et al., 2020). For instance, kasugamycin is recommended for the control of bacterial diseases of several crops, including those caused by Xanthomonas spp., as for example bacterial blight of passion fruit caused by Xanthomonas axonopodis pv. passiflorae, bacterial leaf spot in melon and watermelon caused by Xanthomonas campestris pv. cucurbitae, and bacterial spot in solanaceous plants caused by Xanthomonas vesicatoria (Agrofit, 2020). From our study, the results are important contributions to a better understanding of the bacterial leaf streak, in particular for the lack of control of this bacterial disease by foliar applications of some chemicals regularly used in the management of fungal diseases in corn fields.

Conclusion

The RL1, RL2, XVV1, and XVV2 strains of *Xanthomonas vasicola* pv. *vasculorum* have differences in sensibility to chemical products. Kasugamycin, copper sulfate, copper oxychloride, copper hydroxide, cuprous oxide, bioactive copper, and mancozeb inhibit the growth of the bacterial strains. In contrast, chlorothalonil and methyl thiophanate

fungicides do not have any activity against the strains of *X. vasicola* pv. *vasculorum* tested. Under greenhouse conditions, copper oxychloride has been the only chemical that significantly reduces the incidence and severity of bacterial leaf streak on corn. However, this copper compound may show phytotoxicity to the corn plants.

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